Dissecting emotion : towards a functional neuroimaging probe for affective disorders

Wright, Paul

Awarding institution:
University of Florida

DISSECTING EMOTION: TOWARDS A FUNCTIONAL NEUROIMAGING PROBE FOR AFFECTIVE DISORDERS

By

PAUL WRIGHT

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2006

Dedicated to my parents, who kept me curious about how things worked and what things meant.

ACKNOWLEDGMENTS

I thank my committee members for their different contributions to my apprenticeship: to Yijun Liu for modeling vision, to Christiana Leonard for modeling rigor, to Dawn Bowers for modeling optimism, and to Russell Bauer for modeling practical insight.

Special thanks are due to Andy James for showing me it can be done, to Jason Craggs for helping me overcome inertia, and to Jessica Couch for keeping me moving. I also thank Emmanuel Mennonite Church for their moral and spiritual support.

TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

DISSECTING EMOTION: TOWARDS A FUNCTIONAL NEUROIMAGING PROBE FOR AFFECTIVE DISORDERS

By

Paul Wright

December 2006

Chair: Yijun Liu
Major Department: Medical Sciences--Neuroscience

The goal of this research was to develop a functional magnetic resonance imaging paradigm for use in investigating major depressive disorder. Functional neuroimaging studies of depression have reported altered resting brain metabolism and altered responses to simple emotion paradigms in the amygdala, ventral prefrontal cortex, and anterior cingulate cortex. We studied healthy individuals' responses to complex emotion paradigms to attempt to distinguish activity in these regions. We hypothesized that the amygdala mediates "bottom-up" processing driven by emotional stimulus content, and that the ventral prefrontal cortex and anterior cingulate cortex mediate "top-down" processing driven by explicit knowledge or intention. In the first experiment, we tested whether matching faces by emotional expression elicited a bottom-up response in the amygdala. The amygdala responded during matching of both emotional and non-emotional faces, implying that this response was driven by top-down demands of the task. In the second experiment, we measured responses during rating of emotional pictures. The response in the amygdala was greater to unpleasant than pleasant pictures, regardless of rating task: a bottom-up response. The response in the ventral prefrontal cortex was greater to emotion rating than non-emotional rating, regardless of picture content: a top-down response. The anterior cingulate cortex showed weak, mixed bottom-up and top-down responses. This emotion rating

paradigm improves existing approaches to imaging the neural bases of major depressive disorder by eliciting dissociated responses in two regions implicated in depression: the amygdala and orbitofrontal cortex. This paradigm may be used in future studies to investigate in parallel the effects of depression on bottom-up and top-down emotion processing. Future studies may attempt to elicit more specific responses in the anterior cingulate cortex by using paradigms in which emotional stimuli interfere with the performance of cognitive tasks.

CHAPTER 1
INTRODUCTION

Functional neuroimaging has been used to identify brain regions involved in major depressive disorder (MDD); however the individual contributions of these regions to illness are not known. Specific regional responses may be elicited by using imaging task paradigms that dissect the components of emotion processing. The goal of the experiments in this dissertation is to refine the use of functional magnetic resonance imaging (fMRI) as a tool for probing emotion processing in healthy individuals, in order to improve its usefulness for later investigations of affective disorders. The feasibility of dissecting emotion with fMRI was demonstrated by showing that neural responses to emotional pictures in different regions of the brain were associated with different ratings of picture content (chapter 3). We then attempted to refine two existing paradigms reported to elicit distinct neural responses to bottom-up (stimulus-driven) and top-down (knowledge-driven) processing of emotional stimuli. A face-matching task was reported to elicit bottom-up responses in the amygdala that were inhibited during face labeling. We added a novel control condition to the face matching task to test whether the amygdala responded to stimulus content and not to task demands (chapter 4). Rating emotional pictures has also been shown to recruit cortical regions and to modulate limbic responses. We aimed to reproduce this modulation using an optimized paradigm design, where task components were varied at the event level and responses were detected using factorial analysis (chapter 5). The findings of these studies are discussed in relation to ongoing functional neuroimaging research on MDD, and future studies are recommended (chapter 6).

Specific Aims

Studies of MDD have highlighted three brain regions where resting metabolism or responses to simple emotion tasks were altered: the amygdala, ventral prefrontal cortex (PFC),

and anterior cingulate cortex (ACC) (Davidson et al., 2003;Drevets et al., 1992;Mayberg, 2003;Sheline et al., 2001;Siegle et al., 2002). These regions are posited to be involved respectively in rapid generation (LeDoux, 2000), contextual modification (Rolls, 1999), and explicit appraisal (Lane et al., 1997a) of emotional responses. The goal of the experiments presented in this dissertation is to identify an emotion paradigm that can elicit dissociable responses in limbic and cortical regions to bottom-up and top-down emotion processing, and that can demonstrate modulation of bottom-up responses under different top-down conditions.

Aim 1. Assess the validity and reliability of the amygdala response to a face matching paradigm. Verbal labeling of emotional stimuli is hypothesized to inhibit emotional feelings. A previous study using emotional faces compared verbal labeling with matching faces by their expression (Hariri et al., 2000). During labeling, responses were decreased at the amygdala and increased in the ventral prefrontal cortex. The response to face matching was hypothesized to represent associative (or bottom-up) processing of emotion; however, the task design confounded emotional content with task performance. In order to test the hypothesis that the amygdala response reflected bottom-up processing, we compared face matching with a novel control task in which neutral faces were matched by identity. In order to assess the reliability of the amygdala response, we investigated changes in the response over repeated presentations of the task.

Aim 2. Dissociate the neural correlates of top-down and bottom-up emotion processing using a picture rating task. Responses to emotional stimuli may also be inhibited by tasks requiring that feelings be explicitly attended and appraised. Rating emotional pictures has been shown to recruit the ACC and modulate the response in the amygdala (Lane et al., 1997a;Taylor et al., 2003). We attempted to reproduce these results using an optimized paradigm design. Stimulus content and rating task instructions were randomized at an event level in order

to eliminate expectancy effects. Factorial analysis of the neural responses was used to identify main effects of bottom-up processing of stimulus content, top-down processing of task demands, and their interaction.

Components of Emotion Processing

It is not yet known whether specific symptoms of depression may be linked with specific components of emotion processing. The symptoms of major depressive disorder may be grouped into attentional or cognitive symptoms, such as apathy, psychomotor retardation, impaired attention, and executive dysfunction, and vegetative symptoms, such as disordered sleep, disordered appetite, and endocrine disturbances. It has been proposed that cognitive and vegetative symptoms are mediated respectively by hypoactivity in dorsal, cortical brain regions and by hyperactivity in ventral, subcortical brain regions (Mayberg, 1997). These two circuits may respectively mediate the top-down effects of cognitive behavioral therapy and the bottom-up effects of antidepressant medication (Goldapple et al., 2004). The chronic negative mood effects of MDD may be probed by measuring their influence on acute emotion processing. For example, individuals with a sad mood are more likely to attend to and later to recall sad emotional stimuli (Eysenck, and Keane, 2000). Chronic alterations in cortical and subcortical circuits may be probed separately by measuring acute responses to bottom-up and top-down components of emotion processing.

The production and regulation of emotion is complex. The selection of theories below provide the basis for the working hypotheses use in the current research. Passer and Smith define emotions as "positive or negative *feeling* (affect) states consisting of a pattern of *cognitive*, *physiological*, and *behavioral* reactions to events that have relevance to important goals or motives" (Passer, and Smith, 2001). The physiological reaction to an emotional event was emphasized by the 19th century psychologist William James, who proposed that, "*the bodily*

changes follow directly the perception of the exciting fact, and that our feeling of the same changes as they occur **IS** *the emotion*" (James, 1884, original emphasis). These bodily changes include muscle movement, such as shivering, and visceral responses or autonomic responses, such as heart palpitations. Despite James' emphasis on the physiological reaction, he assumed that when faced with an emotional event, perception and appraisal of the event preceded the generation of a bodily response (Ellsworth, 1994). Furthermore, he proposed that bodily signals must recombine in the brain with a representation of the "exciting fact" in order for an emotion to be felt. Later researchers debated the relative influence of immediate perception and deliberate appraisal upon the behavioral and physiological signs of emotion.

Zajonc proposed the affective primacy hypothesis, according to which events may be appraised emotionally without conscious awareness (Zajonce, 1980, quoted in Eysenck & Keane, 2000). He supported his hypothesis by investigating the behavioral effects of subliminally presented emotional stimuli. Individuals viewing subliminal emotional faces followed by Chinese pictograms rated pictograms preceded by happy faces as more likable. It is not clear, however, whether the change in liking score was accompanied by any feelings about the Chinese pictogram. Lazarus emphasized how emotion was influenced by the conscious appraisal of events (Lazarus, 1982, quoted in Eysenck & Keane, 2000). He showed that physiological responses to a distressing movie of a surgical procedure altered according to whether the movie's narration emphasized or downplayed the emotionality of the events. It has been noted, however, that the movie's narration itself may be considered an emotional stimulus. Although Zajonc and Lazarus present apparently opposing theories, evidence from studies of fear conditioning in rats suggests that emotional behavior may be produced by two parallel appraisal systems. LeDoux identified a fast and a slow route by which sensory stimuli could reach the amygdala and thus

evoke bodily responses. He used this evidence to draw together the theories of Lazarus and Zajonc, stating:

> The activation of the amygdala by inputs from the neocortex [slow route] is … consistent with the classic notion that emotional processing is postcognitive, whereas the activation of the amygdala by thalamic inputs [fast route] is consistent with the hypothesis, advanced by Zajonc (1980), that emotional processing can be preconscious and precognitive. (Quoted in Eysenck & Keane, 2000, p.493)

Thus there is evidence for two anatomic routes for emotion processing. Appraisal theorists later proposed that the components of emotional processing do not follow one another in linear order, but evolve in parallel (Ellsworth, 1994). Ellsworth states, "Neither interpretation, nor bodily feedback, nor subjective experience comes first; at the very most, one can talk about which of these complex temporal processes starts first." If these components of emotion processing are indistinguishable temporally, they might be distinguishable spatially by identifying distinct anatomical correlates of each component. In this dissertation, the neural correlates of two levels of emotion processing are operationally defined:

- **Bottom-up processing** – responses that are determined by the emotional content of stimuli, independently of task demands.
- **Top-down processing** – responses that correlate with the explicit appraisal (naming or evaluation) of perceived or experienced emotion.

It is not clear how these two levels of emotion processing are linked to specific symptoms of MDD. Such a link may be found by identifying brain regions that share a common association with specific components of emotion processing and with specific symptoms of depression. Functional imaging studies of MDD therefore provide the anatomic targets for the emotion paradigms used in this dissertation.

Brain Regions Implicated in Depression

Early studies of resting brain metabolism in patients with MDD measured cerebral glucose metabolism using positron emission tomography (PET), and reported increased metabolism in

the amygdala and ventral PFC (Drevets, 1998). Amygdala metabolism correlated with symptom scores and with plasma cortisol (a hormone associated with response to stress) during depressive episodes. Later fMRI studies extended these findings. The amygdala response was increased in patients viewing pictures of fearful faces, and decreased following successful medication (Sheline et al., 2001). Because the emotional stimuli were presented sufficiently rapidly to prevent their conscious perception, the increased response may reflect altered implicit emotion processing in MDD. However, MDD may also alter top-down processing in the amygdala. The amygdala response to unpleasant emotional words lasted around 10 seconds in controls, but in patients lasted around 25 seconds (Siegle et al., 2002). Because response duration correlated weakly with self-report measures of rumination, the authors associated these changes with prolonged elaborative processing of emotional information. Rumination may also underlie increased metabolism in the PFC, as explained by Mayberg:

> Frontal hyperactivity is now viewed as an exaggerated or maladaptive compensatory process resulting in psychomotor agitation and rumination, serving to over-ride a persistent negative mood generated by abnormal chronic activity of limbic-subcortical structures. In contrast, frontal hypometabolism seen with increasing depression severity is the failure to initiate or maintain such a compensatory state. (Mayberg, 2003, p. 197)

This view is supported indirectly by an fMRI study of patients viewing pleasant emotional pictures (Mitterschiffthaler et al., 2003). Patients lacked the response seen in controls in the medial PFC, but had increased responses in the ventrolateral PFC. Because previous studies showed overlapping responses in the ventrolateral PFC to cognitive and emotional tasks (Drevets, and Raichle, 1998a), the authors suggested that responses to pleasant pictures in this region may reflect an attempt to experience positive emotion.

A compensatory process may also involve the ACC. Studies of resting metabolism in patients with MDD reported different responses in three regions of the ACC, inferior, superior, and anterior to the genu of the corpus callosum. Metabolism in the subgenual ACC is increased

in MDD and during induced sad mood (Mayberg, 1997). In patients who respond to medication, metabolism decreases in the subgenual ACC and increases in dorsal regions, including the supragenual ACC. These changes were replicated in two studies of primary unipolar depression (Kennedy et al., 2001;Mayberg et al., 2000), one of Parkinson's disease patients with secondary depression (Stefurak et al., 2001), and one of patients responding to placebo treatment (Mayberg et al., 2002). Metabolism in the pregenual ACC was unaffected by medication, but pretreatment metabolism in this region distinguished patients who later responded to medication from those who did not (Mayberg et al., 1997). This finding was replicated in an fMRI study of patients with MDD (Davidson et al., 2003). The response to unpleasant pictures at baseline predicted symptom scores following eight weeks of medication. Furthermore, cognitive-behavioral therapy (CBT) increased metabolism in the pregenual ACC, suggesting that this region plays an important top-down role in recovery form depression (Goldapple et al., 2002). The response in the pregenual ACC to mood challenge is greater in remitted depression patients then in healthy controls or patients with active depression (Liotti et al., 2002). This altered response in the absence of treatment supports a protective role for this region.

Anatomical Background

The findings reviewed above suggest that the ventral PFC and pregenual ACC mediate top-down compensation in MDD that may modulate increased bottom-up processing in the amygdala. In general, animal studies, lesion studies, and functional imaging studies agree, describing the roles of the amygdala in forming simple emotional associations, the orbitofrontal cortex in contextual appraisal of emotion, and the anterior cingulate cortex in emotionally guided behavior. An additional region, the insula, is associated with awareness of visceral and autonomic sensations.

The Amygdala

The amygdala appears to be involved in generating rapid but rough responses to stimuli that have emotional value. Its role in mediating fear behaviors is often emphasized, although it also responds to rewarding stimuli.

Anatomy. The amygdala is an almond-shaped nucleus in the medial temporal lobe, anterior to the hippocampus. It shares reciprocal connections with many cortical and subcortical regions (Aggleton, and Saunders, 2000). Its inputs arrive principally at its lateral and basolateral nuclei and are passed, directly or indirectly, to the central nucleus for output. Of particular interest are cortical afferents from insula, temporal and anterior cingulate cortices and from the dorsolateral, medial and orbital prefrontal cortices. The amygdala sends efferents to each of these regions, and back-projections to the occipital and temporal cortices to modulate visual processing. Its subcortical outputs include the tail of caudate, ventral putamen and nucleus accumbens as well as one-way efferents to the mediodorsal thalamus and reciprocal connections with the entire hypothalamus. It receives inputs from the midline thalamus and medial pulvinar as well as various brainstem nuclei.

Animal studies. LeDoux has described in great detail the involvement of the amygdala in fear conditioning in rats (LeDoux, 2000). In his model, fear is operationally defined as defensive posturing in response to an aversive stimulus, such as an electric shock (LeDoux, 2000). Rats can be conditioned to display fear responses to a neutral stimulus (e.g., a tone) by pairing it with the unconditioned stimulus (shock). These conditioned responses are impaired if the amygdala or its connections are damaged. Emotional information may reach the amygdala through a fast, subcortical pathway involving the collicular visual system, or through a slow, cortical pathway, involving occipital and temporal cortex. Single cell recordings in monkeys have implicated the amygdala in responses to both aversive and rewarding conditioning (Rolls, 1999). Some

amygdala neurons responded exclusively to rewarding food-conditioned stimuli or to aversive fear-conditioned stimuli, and others responded to both. Although amygdala neurons rapidly alter their firing patterns to detect conditioned stimuli, these associations appear to be inflexible. More flexible, context-dependent associations may be learned by the orbitofrontal cortex (see below). Some neurons in the monkey amygdala respond selectively to faces, demonstrating a role for the amygdala in responding to socially salient visual cues (Leonard et al., 1985). Monkeys with bilateral amygdala lesions are emotionally unresponsive; they show no fear of snakes or humans and eat items not usually used as food.

Human lesion studies. Humans rarely have lesions confined to the amygdala. Injuries or damage from encephalitis often affect the hippocampus and temporal lobe as well (Aggleton, and Saunders, 2000). There is no single, clear symptom of amygdala damage. It is apparent, however, that such lesions rarely cause cognitive deficits, but generally cause changes in emotionality. Extensive lesions involving the amygdala are associated with Kluver-Bucy syndrome, which includes symptoms of blunted emotions, hypersexuality and hyperorality (placing non-food objects in the mouth). Bilateral amygdala lesions are consistently associated with impaired recognition of facial expressions of fear (Adolphs et al., 1994;Broks et al., 1998;Sprengelmeyer et al., 1999;Young et al., 1995) and occasionally associated with impaired recognition of vocal expressions of fear (Scott et al., 1997).

The Insula

The insula has been described as sensory cortex for the viscera, and may be involved in processing the bodily responses involved in emotion (Adolphs, 2002). This region is notably associated with the emotion disgust, but is also involved in pain and awareness of visceral sensations (Critchley et al., 2002).

Anatomy. The insula is located inside the Sylvian fissure, and is so-called because is completely concealed (insulated) by the temporal and frontal lobes. It is divided from anteroventral to posterodorsal into agranular, dysgranuar, and granular regions (Mesulam, and Mufson, 1982a). The insula receives input from all five sensory modalities, and shares reciprocal connections with the amygdala, lateral orbital cortex, and ACC (Mufson, and Mesulam, 1982). Limbic connections, as well as olfactory, gustatory, and autonomic connections, are particularly extensive in the anteroventral insula (Mesulam, and Mufson, 1982b). Mesulam and Mufson suggest that the insula's connections to the amygdala allow visceral input to the limbic system, and furthermore that its common connectivity patterns with the lateral orbital cortex identify these two regions as part of an integrated paralimbic unit.

Human lesion studies. The insula is associated with impaired recognition of disgust. A patient with selective damage to the right insula and putamen was impaired at recognizing disgust in two sets of face stimuli, and in two sets of vocal stimuli (Calder et al., 2000). Despite being able to recognize disgust conveyed in complex scenes, he scored low on questionnaires measuring the experience of disgust. Another patient with extensive lesions was impaired at recognition of all static emotional stimuli, but could recognize emotions acted out or described in stories, with the exception of disgust (Adolphs et al., 2003). Adolphs et al. suggested that the processing of acted-out emotions bypassed the damaged limbic and ventral prefrontal regions, and relied upon parietal and dorsal prefrontal pathways. They also suggested that recognition of emotion depended upon regions representing somatic states, and that this patient's selective impairment at recognizing disgust stemmed from his selective lesion of this region of somatosensory cortex, with sparing of the more dorsal postcentral regions. Thus the insula

appears to be particularly important in the sensory experience of emotion, and in disgust in particular.

The Ventral Prefrontal Cortex

The ventral PFC is implicated in the flexible association of stimuli with reward and punishment, social functioning, and regulation of mood. A region of the ventral PFC, the orbitofrontal cortex, is heavily connected with the amygdala and may be involved in contextual fine-tuning of primitive emotional signals from the amygdala. Unreferenced data in this section is taken from *The Orbitofrontal Cortex and Reward* (Rolls, 2000).

Anatomy. The orbitofrontal cortex is located on the ventral surface of the frontal lobes, adjacent to the orbits of the eyes. The orbitofrontal cortex receives input from primary gustatory, olfactory, auditory, and somatosensory cortices. The inputs of primary relevance to the current research, however, arise from multiple stages along the ventral visual pathways in the temporal lobe, which are involved in object recognition, and in particular face recognition. The orbitofrontal cortex also receives strong inputs from the amygdala and from the mediodorsal nucleus of the thalamus. Its outputs include back-projections to the ventral visual pathways and outputs to the amygdala, ACC, lateral hypothalamus and ventral striatum.

Animal studies. The orbitofrontal cortex was first associated with reward with the discovery of neurons with selective responses to taste, a primary reinforcer (that is, certain tastes can be rewarding or punishing, such as sweet or sour). The taste reward neurons' activity was enhanced by hunger, and specific to the type of reward, in that a monkey fed to satiety on bananas would maintain an orbitofrontal response to the sight of peanuts while the neurons tuned to bananas ceased responding. When a food reward was associated with a visual stimulus, orbitofrontal neurons responded to that stimulus, provided the animal was hungry. Certain orbitofrontal neurons responded to the reward value of stimuli, even after their associations were

reversed in a visual discrimination reversal task. In this task, food was paired initially with a square symbol, and after reversal was paired with a triangle. Orbitofrontal neurons responding initially to the square rapidly adapted to respond to the triangle after reversal. Some neurons responded to non-reward when reward was expected, for example after the switching described above. Other non-reward neurons responded selectively to removal or termination of a reward, possibly enabling a context-specific response. Certain orbitofrontal neurons in macaques carry information about faces. They distinguish both expression and identity, probably receiving this information from neurons in the temporal visual cortex, consistent with the connections described above.

Human lesion studies. Human with orbitofrontal lesions tend to be euphoric, and have difficulty in planning and social functioning. The classic prefrontal lesion patient is Phineas Gage, a 19th century railway foreman who survived the passage of an explosive-driven railroad spike into his left cheek bone and out of the top of his head, passing through his prefrontal cortex. Previously hard-working and respected, Gage began to neglect his work duties and his marriage, engage in drinking and brawling. More recent studies have shown that patients with prefrontal lesions and impaired social functioning perform badly on certain tasks. When human subjects were asked to perform a visual discrimination reversal task similar to the one above, subjects with ventral prefrontal lesions made more errors than controls, apparently because they were less able to correct their behavior. Test performance correlated with measures of social impairment. Similar patients were impaired at a gambling task in which two decks of cards were presented, one that gave large rewards but larger penalties and another that gave small rewards but smaller penalties (Bechara et al., 1994). Patients with ventral prefrontal lesions were more likely than controls to pick the high-reward deck even when net gain was greater with the small-

reward deck. They could apparently discern only the short-term positive, not the long-term negative consequences of their decision. Some patients with orbitofrontal lesions were unable to recognize emotion in facial expressions and/or speech (Hornak et al., 1996). The latter deficits were distinct from impaired visual discrimination reversal.

The Anterior Cingulate Cortex

The anterior cingulate cortex is closely related both to medial prefrontal cortex and to motor cortex. It is associated with numerous functions in addition to emotion processing, including detection of pain and control of attention (Kerns et al., 2004;Vogt, 2005).

Anatomy. The cingulate gyrus forms a semi-circular belt on the medial surface of the cortex, surrounding the corpus callosum. The cingulate gyrus was first associated with emotion when Papez included it in his famous emotion circuit (Papez, 1937). Papez postulated that just as the striate cortex was considered to be receptive cortex for visual signals from the retina, the cingulate gyrus may be considered to be receptive cortex for emotional signals from the hypothalamus. He also saw the cingulate gyrus' extensive cortical outputs as a means by which emotion could color other experiences, and cortical inputs to the cingulate, as a means by which emotion could be generated by "psychic processes", as an alternative to visceral inputs. Papez described the region as "the seat of dynamic vigilance by which environmental experiences are endowed with an emotional consciousness." The anterior cingulate cortex receives afferents from the medial orbitofrontal cortex, the amygdala, the temporal pole cortex and somatosensory cortex, including the insula (Rolls, 1999). It receives rich dopaminergic innervation from the ventral midbrain (Bannon, and Roth, 1983). Its efferents extend to the periaqueductal gray in the midbrain, the dorsal motor nucleus of the vagus nerve and the ventral striatum and caudate nucleus (Rolls, 1999).

Animal studies. Different behaviors may be associated with afferent signals from different regions of the ACC. The subgenual ACC projects to the medial hypothalamus and ventrolateral periaqueductal gray, whereas the pregenual ACC projects to the dorsal hypothalamus and lateral periaqueductal gray (Ongur, and Price, 2000). Ongur and Price (2000) suggest that these projections allow the subgenual and pregenual regions to evoke coordinated emotional responses, resulting respectively in quiescent or confrontational stances. Shima and Tanji trained monkeys to push or to turn a handle, by associating a reward with the preferred action. Certain neurons in the monkey anterior cingulate cortex responded to decreased reward, but not to constant reward. When the monkeys failed to adjust their behavior in response to reversal, these cells also failed to fire (Shima, and Tanji, 1998). This is similar to the orbitofrontal response to stimulus-reward reversal, but that the association is with action, not a stimulus. Injections of a GABA agonist into the anterior cingulate prevented the monkeys from altering their task behavior in response to changing reward. Thus the error-correcting activity of the anterior cingulate cortex could be interpreted as part of a reward-seeking mechanism.

Human lesion studies. Humans with anterior cingulate strokes appear to lose their initiative, and despite intact cognition and motor function, become quite inactive and rarely even talk (Damasio, and Van Hoesen, 1983). Patients treated for chronic pain with bilateral 5mm lesions of the anterior cingulate cortex report that the pain continues but no longer causes them distress. They also showed less spontaneous behavior compared with controls, producing shorter statements at a written task and producing fewer and simpler models when asked to put together Tinker Toys (Cohen et al., 1999). These findings, along with the animal studies above, suggest that the anterior cingulate cortex, particularly its pregenual region, may mediate the influence of processed emotional information on the adjustment and initiation of motor behavior.

Emotion Processing in Healthy Individuals

In patients with depression, functional imaging studies have pinpointed the amygdala, orbitofrontal cortex, and anterior cingulate cortex as possible sites for deficits. Anatomical and lesion studies have described the amygdala's role in associating emotional experiences with perceived stimuli, the orbitofrontal cortex's role in assigning context-dependent emotional value to stimuli, and the anterior cingulate cortex's role in selecting actions based on their consequences' anticipated value. These regions have been investigated in healthy humans using PET and fMRI paradigms involving the perception and evaluation of emotional stimuli. Although a wide variety of emotional tasks have been used, this dissertation focuses on those that use visual stimuli to elicit emotion processing. Standardized sets of visual stimuli are available with rigorous descriptions of their content, allowing creation of precisely-defined emotional and control stimulus sets, and increasingly the likelihood of reproducible results across experiments. Using visual stimuli to elicit emotion processing allows precise stimulus timing, improving the detection of the resulting neural responses. The Pictures of Facial Emotions consists of faces expressing happiness, sadness, fear, anger, disgust, and surprise (Ekman, and Friesen, 1976). These expressions are reliably recognized across cultures, implying that they are not socially learned, but may represent basic categories for the social communication of emotion (Ekman, 1982). The International Affective Picture System consists of emotional scenes with a wide variety of content, eliciting a range of emotional responses (Center for the Study of Emotion and Attention [CSEA-NIMH], 2001). These pictures have been rated along three dimensions of emotion: valence (from pleasant to unpleasant), arousal (from calm to excited), and dominance (from controlled to in control). Ratings for pictures at either end of the valence scale tend also to have high arousal ratings. These rating scales have been validated using physiological measures of emotional responses (Lang, 1995). Eye blink

responses to auditory startle are increased by unpleasant pictures and decreased by pleasant pictures. Skin conductance responses to pictures are proportionate to their arousal scores. Functional imaging studies have used both facial expressions and emotional scenes to investigate the neural correlates of bottom-up and top-down processing of emotion.

Processing Emotion in Facial Expressions

Facial expressions enable the social communication of emotion. Neural responses to faces cannot be assumed to correlate with the experience of emotion, but may correlate with automatic (bottom-up) *perception* of emotion, or explicit (top-down) *recognition* of emotion (Davidson, and Irwin, 1999). Facial expression recognition is impaired in patients with MDD, supporting the use of face stimuli in imaging investigations of MDD (Gur et al., 1992). Furthermore, recent studies have shown that brain regions that respond to facial expressions of disgust or pain overlap with brain regions that respond to the experience of disgust or pain (Singer et al., 2004;Wicker et al., 2003). This evidence suggests that the neural correlates of emotional communication and emotional experience may at least partly overlap, further supporting the use of face stimuli in imaging studies of affective disorders.

Lesions studies have implicated the amygdala in the perception of fear in facial expressions (Adolphs et al., 1994). This role was confirmed in numerous fMRI studies, which reported a bottom-up, stimulus-driven response in the amygdala to faces expressing fear and other salient emotions. The amygdala responded to fearful facial expressions regardless of whether attention was paid to emotion, to the gender of the faces (Morris et al., 1996;Winston et al., 2002;Winston et al., 2003), or to the properties of another stimulus (Anderson et al., 2003;Vuilleumier et al., 2001). The amygdala also responded when emotional faces were displayed very briefly (~30 ms) and rapidly replaced with a neutral face, a technique called backwards masking that prevents conscious awareness of the stimulus (Morris et al.,

1998;Whalen et al., 1998b). Other studies showed that the amygdala response may be modulated by top-down effects, reporting increased responses during explicit recognition of happy and disgusted faces (Gorno-Tempini et al., 2001), and decreased responses during explicit recognition of happy and angry faces (Critchley et al., 2000), both compared with gender recognition. Top-down modulation of the amygdala may be mediated by the prefrontal cortex.

Patients with lesions in the ventral PFC have general social impairments (Rolls, 1999) and specific impairments in recognizing facial expressions (Hornak et al., 1996). Comparing emotion recognition with gender recognition elicited specific responses to emotion recognition in prefrontal regions (Gorno-Tempini et al., 2001;Winston et al., 2003) and elicited amplified responses to emotional faces in the fusiform gyrus and superior temporal sulcus (Critchley et al., 2000;Winston et al., 2002). These variable results may reflect multiple strategies for emotion recognition: some participants may hold in mind verbal labels for the candidate emotions in order to guide their response, others may use a non-verbal strategy, such as holding in mind a visual example of each facial expression. Later studies investigated the role of the ventral PFC in emotion recognition by compared verbal and non-verbal emotion recognition. One study compared facial expression matching (a perceptual task) with facial expression labeling (an intellectual task) (Hariri et al., 2000). The amygdala response was lower during labeling then matching, and correlated inversely with activity in the right ventral PFC, suggesting that verbal judgments of emotion recruit top-down inhibition by the PFC of bottom-up processing in the amygdala. A subsequent study compared verbal and facial cues in a delayed match to sample task involving emotion or gender judgments (Narumoto et al., 2000). This study found no amygdala response, and reported right prefrontal activation to both verbal and non-verbal emotion judgment tasks. The difference in amygdala responses between these tasks may be

because Hariri et al. (2000) displayed only fearful or angry faces whereas Narumoto et al. (2000) showed all six of Ekman's basic facial expressions, counterbalancing the valence of the stimuli. The prefrontal response to the non-verbal task in Narumoto et al. (2000) may be driven by the requirement to maintain a representation of the emotional information from the cue in working memory, whereas in the task employed by Hariri et al. (2000) all stimuli are present simultaneously.

Because the Hariri task may elicit top-down modulation by the ventral PFC of bottom-up responses at the amygdala, we chose to investigate this task further. By requiring participants to read verbal labels in every trial, the labeling condition of the Hariri task is likely to limit participants' recognition strategy to verbal processing, and thereby elicit top-down processing. The matching condition of the Hariri task has been shown to activate the amygdala reliably in studies looking at the influence on the amygdala of genetics (Hariri et al., 2002b;Pezawas et al., 2005), drugs (Hariri et al., 2002a;Tessitore et al., 2002), and aging (Tessitore et al., 2005). However, the matching condition is more likely to involve knowledge-based processing than other control tasks, and may not elicit a true bottom-up response. Hariri et al. (2000) claimed that participants do not match facial expressions using covert verbal labeling, but using perceptual cues such as wide eyes or a furrowed brow. Perceptual cue matching may represent intentional, knowledge-based processing in pursuit of task demands, or top-down processing Therefore, in order to distinguish facial feature matching from emotion processing, and presumably thereby to dissect an emotional response at the amygdala, we modified the Hariri task to include an intermediate control condition in which neutral faces were matched by identity. This experiment tested the validity of using the face matching task to investigate bottom-up emotion processing in

the amygdala by examining whether the amygdala response is driven by emotional content or task demands.

Processing Emotion in Complex Scenes

The emotional scenes in the International Affective Picture System (IAPS) differ from the Ekman faces in several important respects. First, they are complex, and differences in emotion are conveyed not by small shifts in facial configuration, but by multiple components of the image. Second, whereas faces represent social signals of emotion (eliciting emotional perception), scenes are more likely to evoke emotion directly, eliciting emotional experience. Whereas top-down processing of faces involves recognition of an emotional category, top-down processing of scenes is more likely to involve the appraisal of internal feelings. Third, the subjective ratings categorizing the Ekman faces are based on specific categories, but the ratings of IAPS pictures are based on general dimensional scores.

As with emotional faces, viewing unpleasant scenes elicits a response in the amygdala (Irwin et al., 1996;Taylor et al., 1998). Studies using IAPS pictures also report greater responses to emotional than neutral scenes in the ventral temporal cortex (Lane et al., 1997b;Lang et al., 1998). This response appears to be specific to unpleasant scenes (Lane et al., 1997b) and correlates with activity in the amygdala (Sabatinelli et al., 2005). Increased ventral temporal responses to unpleasant pictures are thought to be driven by back-projections to this region from the amygdala. Interestingly, in a study of patients with MDD, the contrast between responses to unpleasant and neutral scenes was greater in patients than in controls in the ventral temporal cortex but not in the amygdala (Davidson et al., 2003). The responses in the amygdala and ventral temporal cortex correlate with arousal scores, and thus appear to reflect bottom-up responses driven by stimulus content (Sabatinelli et al., 2005).

The preliminary data presented in chapter three used IAPS pictures to dissociate a response in the ventral temporal cortex to arousal from a response in the insula to disgust. By using both dimensional and categorical ratings to describe stimulus content, this study dissected two components of bottom-up processing. Previous studies reported dissociated responses in the the amygdala to fearful faces and in the insula to disgusted faces (Phillips et al., 1997;Sprengelmeyer et al., 1998). A study of patients with obsessive-compulsive disorder supported these findings by showing that in patients, the insula response was greater to disgust-inducing IAPS pictures than fear-inducing pictures (Shapira et al., 2003). However, two other studies using IAPS pictures reported equal activation of the insula to both disgust- and fear-inducing pictures (Schienle et al., 2002;Stark et al., 2003). The stimuli used to elicit disgust differed between studies, the former using only pictures of spoiled food, garbage, and other contaminants, and the latter using in addition pictures of injuries, tumors, and other mutilations. In the study reported in chapter three, we examined separately the neural responses to pictures of contamination and pictures of mutilation, comparing both with pictures that elicit fear. Because contamination pictures elicit low arousal scores and mutilation pictures elicit high arousal scores, this study allowed us to investigate separately the neural correlates of the emotional category disgust, and the emotional dimension arousal.

Top-down processing of IAPS pictures has been investigated both using a labeling task, as described above, and using emotional rating tasks. Hariri et al. (2003) repeated their matching and labeling study using IAPS pictures. In this study, the matching task involved identifying identical threatening photographs (for example, a picture of a gun), while the labeling task involved choosing selecting between the verbal descriptors “natural” and “artificial”. The amygdala response was larger for matching than labeling, while a larger response to labeling was

found at the ventral prefrontal cortex (BA 47) (Hariri et al., 2003). The responses of these two regions were negatively correlated. While these responses echoed those in Hariri et al. (2000), the second task did not examine top-down processing of emotion. In the original Hariri task, the labels were "angry" or "afraid", whereas in the second task, labeling required semantic processing of non-emotional stimulus content. Replicating the face matching and labeling task using emotional scenes is hindered by scenes' complexity. While different face pictures within an emotional category share general features, different scenes eliciting, for example, fear may vary widely in their visual features, making matching more difficult. Also, while categorical labels for facial expressions of emotion are widely recognized, categorical labels for emotional scenes have not been established. Perhaps for these reasons, studies of top-down processing of emotional scenes usually require participants to rate the scenes simply as pleasant or unpleasant.

In the earliest picture rating study, participants viewed blocks of mixed unpleasant and neutral pictures during and rated either whether each picture was pleasant or unpleasant or whether it was indoors or outdoors (Lane et al., 1997a). Emotion rating elicited larger responses than location rating in the anterior cingulate cortex (ACC) and medial prefrontal cortex (PFC). This study could not investigate bottom-up responses because unpleasant and neutral pictures were intermingled. In two subsequent studies, pleasant and neutral pictures were presented separately to identify regions involved in bottom-up responses (Liberzon et al., 2000;Taylor et al., 2003). Liberzon et al. (2000) compared emotion rating with picture recognition, a cognitive task intended to draw attention away from emotion. The right amygdala responded more to unpleasant than neutral pictures, and this contrast was greater during emotion rating than during picture recognition. Taylor et al. (2003) compared emotion rating with passive viewing, in order to test whether top-down processing diminished bottom-up responses. The right amygdala and

insula responded to unpleasant pictures, but the response was smaller during emotion rating than during passive viewing. The opposite effect was seen in the ACC and medial PFC: responses to unpleasant pictures were increased during emotion rating compared with passive viewing. These studies suggest that the amygdala and insula may mediate bottom-up responses to the content of emotional scenes, and that these responses may be modulated by top-down processing mediated by the ACC and medial PFC. Because these studies used block designs, which may confound bottom-up emotion processing with expectation of emotion (a top-down effect), we attempted to replicate these findings using an optimized event-related paradigm. In our task, both emotional and non-emotional rating tasks required ratings on continuous scales: either the pleasantness of the picture, or the frequency of its appearance on television. Stimulus content and tasks instructions were randomized on a trial-by-trial basis, preventing expectancy of emotional content, and equalizing the timing of bottom-up and top-down responses. Factorial analysis was used to separate the main effects of bottom-up and top-down processing and their interaction.

CHAPTER 2
METHODOLOGY

Functional magnetic resonance imaging (fMRI) measures neuronal activity indirectly by detecting changes in blood oxygenation. By localizing these changes during a stimulus or task, fMRI may indicate the neural correlates of that task. FMRI is non-invasive, and has comparatively good spatial resolution. However, the temporal resolution of fMRI is limited by the sluggishness of the blood oxygenation level-dependent (BOLD) response. Furthermore, responses must be detected by comparing signal during an experimental task and a control condition. Therefore, in order to elicit reliable, valid responses, fMRI paradigms must present stimuli with optimal timing, and must compare task conditions that are matched for every cognitive factor but the one being studied. This chapter describes the physical basis for fMRI, the basics of paradigm design, and some advanced techniques for confirming the validity of statistical maps and for investigating responses to interacting cognitive functions. Unreferenced material is taken *Functional magnetic resonance imaging* (Huettel et al., 2004) or from class notes from BCH 6741, Magnetic Resonance Imaging and Spectroscopy, taught by Dr. Thomas Mareci.

How fMRI Works

Magnetic resonance imaging is based on a radio signal produced by excited hydrogen nuclei (spins) in a strong magnetic field. The images produced by MRI are divided into slices, and each slice is composed of units called voxels. A voxel is comparable to a pixel (picture element) in a computer image, but because MRI slices have thickness, their cubic constituents are called ‘volume elements’. The intensity of every voxel in a slice is calculated by decoding a single, complex radio signal that combines the individual radio signals from each voxel. The individual signals are encoded by varying their frequency and phase according to their spatial

locations. Frequency and phase encoding, and the complex calculations used to reconstruct MR images, will not be discussed here. We will assume that MR signal intensity at a given voxel is proportional to the radio signal produced at that voxel. Functional MRI measures signal that is sensitive to changes in blood oxygenation. This signal decays over time, as described in Equation 1.

(1) $S(t) = S_0 e^{-t/T2^*}$

Where S = signal, t = time, S_0 = signal at t=0, and T2* = decay constant.

Equation 1 shows that signal decay rate is exponential, and varies according to the T2* decay constant. T2* describes the combined influence of a number of factors upon signal decay. One factor, T2, describes interactions between the spins, which varies between different tissue types but is essentially constant. In an ideal situation T2* = T2, but in reality T2* is shorter than T2, and real signal decay rates are more rapid than ideal signal decay rates. This is due to the influence of inhomogeneity effects, or local imperfections in the magnetic field. Inhomogeneity may be caused by the presence of paramagnetic material, such as deoxyhemoglobin, or interfaces between air and water. It is the inhomogeneity component that causes T2* to vary over time, which in turn causes the changes in signal seen in fMRI.

The variations in T2* that form the basis of fMRI are caused inhomogeneity produced by deoxygenated hemoglobin. Because the hemoglobin molecule contains an iron atom at its core, its magnetic properties depend on whether the iron is exposed. Oxygenated hemoglobin has a concealed iron molecule and is diamagnetic, causing no magnetic effects. In deoxygenated hemoglobin, the iron is exposed, making the molecule paramagnetic and capable of producing inhomogeneity. The net result is a decrease in MR signal in the region of blood vessels containing deoxyhemoglobin. This effect was described in detail by systematically varying blood

oxygenation in rats (Ogawa et al., 1990). When perfused with oxygenated blood, vessels are indistinguishable on MRI from the surrounding brain tissue, but when perfused with deoxygenated blood, vessels become dark. Ogawa et al. named this effect the blood oxygenation level-dependent, or BOLD, contrast. BOLD contrast was altered by changes in inhaled carbon dioxide, blood glucose level, and level of anesthesia, indicating that it is determined by both cerebral blood flow (supply) and cerebral metabolism (demand). From these data, Ogawa et al. predicted that BOLD contrast could be detectable within physiological parameters for blood oxygenation, and that BOLD MRI could be used as a complement to PET for imaging functional brain activity.

Shortly after the discovery by Ogawa et al., the first fMRI studies appeared. Changes in the BOLD signal were induced in the occipital lobe using an alternating visual stimulus, and in the central sulcus using alternating hand movements (Kwong et al., 1992). While these experiments measured responses to extended periods of stimulation, a later experiment demonstrated that changes in BOLD signal were detectable to visual stimuli lasting only two seconds (Blamire et al., 1992). The change in BOLD signal was delayed about 2-3 seconds after the stimulus, a delayed response known as the BOLD hemodynamic response (HDR). The HDR is thought to reflect an increase in blood oxygen that occurs, after a delay, in response to neural activity.

The BOLD HDR is now known to have reasonably predictable characteristics. It begins to rise about 2-3 seconds after the start of the stimulus, and falls about 10-15 seconds after the end of the stimulus. Thus the temporal characteristics of neural responses are delayed and smoothed over time in the BOLD HRD. However, the characteristics of the BOLD HDR are sufficiently predictable to allow detection of neural events that are less than 10-15 seconds apart. The BOLD responses to two visual stimuli presented two seconds apart appear to add roughly linearly (Dale,

and Buckner, 1997). Subtracting the response to a single stimulus from the response to two stimuli revealed that the remaining response to the second stimulus was comparable to the response to the first stimulus. For most fMRI analyses, it is assumed that BOLD responses are invariant over time, and between brain regions, and that they add roughly linearly. This allows the use of General Linear Modeling, and event-related analyses, as discussed below.

Several studies have sought to explain in detail the relationship between neural activity and the BOLD HDR. This question was addressed directly in a study using monkeys in which BOLD signal and electrical responses were recorded simultaneously (Logothetis et al., 2001). In this study, a novel MRI-compatible electrical recording system was used to demonstrate that increases in the BOLD signal were indeed related to increased electrical activity. Specifically, the main driver of the BOLD HDR was the local field potential. This electrical signal represents the sum of postsynaptic events at the recording site, or the net input to the neuron. A number of theories propose to explain the link between neural activity and increased oxyhemoglobin. If increased cerebral blood flow matched increased neuronal metabolism, then the concentration of blood oxygen would remain constant, and no BOLD response would be detectable. This mismatch between supply and demand that produces the BOLD response may reflect an overcompensation that anticipates future increases in demand, or may be characteristic of anaerobic metabolism. The latter view was proposed by Shulman et al. (2001) in their astrocyte-neuron lactate shuttle model. In this model, anaerobic metabolism is required for the rapid clearance of glutamate from the synaptic cleft following a burst of firing (Shulman et al., 2001). This model supports the findings of Logothetis et al. (2001) because it links the BOLD HDR to postsynaptic activity.

In summary, fMRI generates images of the brain in which the intensity of signal is modulated by changes in blood oxygenation. The BOLD responses to primary visual and motor stimuli occur in well-established visual and motor regions of the brain (Blamire et al., 1992;Kwong et al., 1992). However, the location of BOLD responses probably does not reflect the location of firing neurons, but more likely reflects post-synaptic activity (Logothetis et al., 2001). Despite the sluggish nature of the BOLD response, individual responses appear to add linearly, and thus may be modeled even in response to brief stimuli. A number of experimental designs and analysis techniques have been developed in order to increase certainty that the detected BOLD response reflects task-related brain activity.

Basics of fMRI Paradigm Design

The primary goal when designing an fMRI paradigm is to evoke a response that will be distinguishable from noise. Task-related responses are detected as differences in BOLD signal during task and control conditions, and these differences are typically small, around one percent. Task-related responses must be distinguished from changes in signal due to non-task-related factors (noise). *Thermal noise* or *intrinsic noise* is the unavoidable, random variation in the signal due atomic vibrations within system components. *Scanner drift* is a gradual, monotonic decrease in signal, which may occur due to slow changes in temperature in the scanner's magnetic coils. *Physiological noise* may arise from visceral motility, breathing, or the heart beat. *Motion artifact* occurs due to the movement of the head during scanning. Finally, fMRI signal changes may be due to *non-task-related neural activity*. Thermal noise is unavoidable, and is overcome by choosing a stimulus that will evoke as large a response as possible. Monotonic sources of noise, such as scanner drift, are overcome by repeating the stimulus on/off conditions several times. Thus, task-related signal changes will be distinguishable by their periodic nature. Physiological noise, however, is also periodic. To avoid this noise, the frequency of task

presentation must be chosen such that is distinct from the frequencies at which physiological noise occurs. Practically, this means that periods of stimulation should last between 2 – 30 seconds. Motion artifact is difficult to avoid, but maybe overcome by immobilizing the subject's head. Any surviving motion may be corrected during later analysis, and if the motion is too large, the subject's data are discarded. Since any movement of the jaw increases motion artifact, fMRI tasks involving speech are difficult to analyze, and where possible subjects should make task responses by manual button presses. Non-task-related neural activity is the most difficult variable to control. Analyzing data from a larger group of subjects theoretically maximizes task-related activity, and minimizes non-task-related activity, which is assumed to be different for each individual. It is important to choose the control conditions carefully, so that the comparison used to search from brain activity truly reflects the cognitive functions of interest.

Two types of fMRI paradigms are commonly employed, and both are used in this dissertation. These are the *block design* and the *event-related design*. In the block design, a series of trials of the same type are presented consecutively, followed by a series of trials of another type, or a rest period. Blocks of each stimulus type are usually alternated several times using the so-called *boxcar design*. This repetition avoids false signal detection due to scanner drift. In the event-related design, trials are presented in random order, either mixed together or separated by a period of rest. The advantage of the block design is that it produces a large, easily detectable signal. The disadvantage of the block design is that the subject is able to anticipate upcoming trials, since the series of trials are all alike. The event-related design overcomes this expectancy effect by randomizing trial types. Furthermore, because trials are analyzed individually, event-related designs allow analysis based on the subject's responses to individual trials, such as error rate or response time. The disadvantages of the event-related design are that the BOLD responses

to individual trials are small, requiring many repetitions of each trial type to produce a detectable response, and that the BOLD responses to event-related trials may overlap. One way of overcoming this overlap is to space events > 15 seconds apart. Alternatively, both these hemodynamic issues may be overcome by jittering the timing of event presentation. By jittering the delay between events, the variance in the resulting BOLD HDR becomes larger the more rapidly events are presented (Burock et al., 1998). This increases the detectability of the BOLD response, and allows a greater number of repetitions of each event type. Therefore the block design is appropriate if the neural response to the task should not vary when the trial is expected. The event-related design is preferred when expectancy effects must be avoided, or when the study is investigating differences in neural activity with different responses to the task, such as error trials vs. correct trials.

Basics of fMRI Analysis

What most fMRI analyses have in common is that they create maps of the brain representing differences between the task and control conditions. These differences are usually represented as statistical values resulting from a comparison of the BOLD response to each condition. Plotted as a colored overlay on an anatomic image of the brain, these statistical maps create the well-known colored clusters of "activation" that illustrate functional imaging studies. This section explores how these clusters are generated, in order to show that they do not represent a direct photograph of brain activity, but instead are informed by a series of statistical decisions made by the experimenter.

Before statistical maps are created, fMRI data are preprocessed. In studies of groups of subjects, the images from each subject are oriented within a standard space defined by the midline of the brain, the line between the anterior commissure and the posterior commissure, and the outermost surfaces of the cortex (Talairach, and Tournoux, 1988). The data from each subject

may be corrected for motion. This correction operates by comparing the whole-brain image at each time point with the initial time point, and then minimizing differences using iterated small rigid-body transformations of the later time point. The correction process results in a series of rotation and translation values that allows head movement to be approximated and thus allows the exclusion of data with large motion.

Although early fMRI experiments created statistical maps by applying t-tests to the raw signal during each task condition, the experiments in this dissertation were analyzed by modeling an estimated BOLD HDR. This approach uses General Linear Model (GLM) statistics. The GLM may be applied using the fixed or random effects approaches. Both are used in this dissertation. The fixed effects approach is applicable to small data sets, but is susceptible to one highly-responsive individual dominating the result. The random-effects approach is more conservative, detecting consistent changes across the group, but requires larger sample sizes. In both approaches, the analysis begins by modeling the BOLD HDR to each task condition. Although this modeling approach may approximate a square wave in slow block designs, and thus represents only a slight improvement over the t-test, modeling is particularly important in event-related designs, where there are greater differences between the timing of stimuli and the timing of the modeled BOLD HDR. Based on stimulus timing, the BOLD HDR may be estimated using a standard response function (Boynton et al., 1996). The modeled response, or *reference time course*, may then be fit to the fMRI signal at each voxel. After estimating a baseline value, the magnitudes of the reference time courses for each task condition are varied until the difference between the model and the data is minimized, using the partial least squares approach. The estimate of the response magnitude is called the beta weight. The solution to the general linear model for a given voxel is given in Equation 2.

$$S(t) = \beta_x \,.\, M_x(t) + \beta_y \,.\, M_y(t) + e(t)$$

Where S = MR signal, t = time, β_{task} = beta weight for task x or y, M = modeled hemodynamic response for task x or y, and e = error, or baseline.

The modeled response for each condition is identical for each voxel, being determined by the timing of the paradigm. The error term is the same at each voxel, being based upon the signal during a baseline or resting task condition. Statistical maps are derived from the beta weights.

In the fixed effects approach, a single beta weight (for each task condition at each voxel) is calculated for the whole group. The statistical significance of the response to each task condition (or the contrast between selected task conditions) is calculated using the magnitude of the beta weight (or contrast) and its standard error. The standard error is based upon the differences between model value and actual signal value, or residuals. The mixed effects approach is vulnerable to bias resulting from a strong response from a single individual. This bias is overcome by using the random effects approach, in which individual beta weights are calculated for each task condition at each voxel, one for each subject. The statistical significance of the response to each task condition (or the contrast between selected task conditions) is then calculated by performing a t-test on the sample of beta weights. Although this approach is less susceptible to contamination from one highly-responsive individual, it requires a larger sample size. In the fixed effects analysis, the number of degrees of freedom is determined by the number of time points in the group data. This is the number of subjects multiplied by the number of data points in one fMRI run, which usually is in the thousands. In the random effects analysis, the number of degrees of freedom is determined by the number of subjects only. Thus to reach a given level of significance, a higher statistical score must be obtained in the random effects analysis compared with fixed effects.

In both approaches, statistical scores are overlaid upon an anatomical image of the brain. Typically, the statistical values are represented by a range of colors. To make the map more readable, a threshold is usually applied to eliminate areas where the response was less significant. The choice of statistical threshold is the topic of ongoing debate in the fMRI literature. The main issue involves correction for multiple comparisons. Since a statistical test is applied at every voxel, then for every 100 voxels, 5 will have a significant response at $p < 0.05$. In fact, there are over 10,000 voxels, so we would expect at least 500 false positive voxels. The choice of statistical threshold, and method for correction for multiple comparisons, will be discussed in each study's chapter.

In summary, fMRI analysis produces brain maps of statistical values. In this dissertation, these values are calculated by fitting a model of the hemodynamic response to the data at each voxel, and then performing statistics upon the model. This may be done using a fixed effects approach or a random effects approach. The former is better suited to small sample sizes, but is vulnerable to contamination by a single, highly-responsive individual. The latter is a more conservative approach, requiring larger sample sizes. Because statistical maps represent highly processed information, and because there are multiple opinions about how best to decide which clusters of activation are significant, an important skill in fMRI analysis is interpretation.

Interpreting fMRI results

As noted above, there are various sources of noise in fMRI data. Although a statistical map may reveal clusters of significant signal change, these may or not reflect the neural correlates of the task condition being tested. This is immediately obvious when "activation" is seen outside the brain. Significant signal changes outside the brain may occur due to task-related head or eye movement. The former is particularly prevalent at the borders of light and dark regions, such as the skull, the edges of the brain, or large sulci. It may be possible to identify motion and other

physical noise effects (like arterial pulsation) by examining the BOLD response at each cluster of activation. Unlike physical noise effects, however, non-task-related neural activity have not have a pattern that can distinguish it from task-related neural activity. It can only be eliminated by careful task design and by having an *a priori* hypothesis about the brain regions involved in the task. The approaches given below are each applied in at least one of the experiments in this dissertation in an attempt to increase the certainty that the apparent fMRI activations indeed reflect use of the hypothesized cognitive function.

Examining the BOLD Response to Exclude False Activation

As explained above, the clusters of activation illustrated on fMRI statistical maps are indirect indicators of brain activity. In order to confirm whether the underlying MR signal changes support the conclusion of the statistical tests, a retrospective examination of the BOLD HDR at each cluster of statistical activation may be performed. This is achieved by averaging the responses to each task condition within subjects, and then averaging the responses across subjects. The MR signal for each response is normalized to a percentage change from baseline before averaging. In block design paradigms, the response should rise and fall within the time window for averaging the BOLD HDR. In event-related paradigms, the time window for averaging the BOLD HDR to a given event may include one or more subsequent events. In order to minimize the influence of subsequent events, the delay between events is randomly jittered. Once the group average BOLD HDR has been calculated for each task condition, certain judgments may be made about the region where the response occurred.

The expected BOLD response to a stimulus is a smooth curve that rises approximately 2-3 seconds after stimulus onset, and then falls approximately 10-15 seconds after the stimulus ends. In reality, there is a large amount of variability in the BOLD responses between individuals, and within individuals, both in time (between repeated blocks, or different scanning sessions) and

between brain regions (Aguirre et al., 1998;Menz et al., 2006;Miezin et al., 2000). One consequence of this is that the BOLD HDR may reveal patterns in the responses to different task conditions that are missed by the GLM, because of discrepancies between the modeled and actual response. Post-hoc analysis of the average BOLD response may confirm whether these patterns are statistically significant.

Several unexpected patterns of BOLD response may be seen. First, the signal may be inverted. An apparent "activation" on a statistical map may be due to a decrease in BOLD signal during the control condition, while signal during the task does not change. Deactivation during task performance may implicate a brain region in cognitive processes that occur during the control condition. This effect has been reported consistently in a set of brain regions including the anterior and posterior cingulate cortices, and the bilateral angular gyri (Shulman et al., 1997). Because these deactivations are deeper with increasingly demanding tasks, they are taken to represent mental functions performed at rest that require attention (McKiernan et al., 2003). A second unexpected pattern of BOLD response is a steadily rising curve. This may represent changes in tonic activation of a brain region, or may represent noise effects, such as task-related head motion. One indication that a statistical activation is due to a noise effect is a high degree of variability in the BOLD response, as measured by the standard error of each time point. Further examination of individual subjects' responses may reveal that a large jump in signal in one individual led to a statistically significant result.

Using Control Conditions to Test Specific Cognitive Components

Having ruled out physical noise effects, the investigator must ask whether a particular cluster of activation corresponds to a particular cognitive function. The earliest fMRI experiments compared simple visual stimuli with a resting condition: a blank screen. In a more complex task, for example a face recognition task, a more detailed control condition may be

required. For example, to look for specific neural correlate of face recognition, an experiment may include a condition in which subjects must recognize common household objects. In addition to determining the response to face recognition and object recognition versus rest, the two conditions may be compared with each other. This is achieved in the GLM approach by looking for significant differences between the beta weights for each task condition. This *subtractive method* assumes that subtracting object recognition from face recognition eliminates any brain regions that respond equally to both tasks, and are therefore not specifically activated by face recognition. Although the subtractive method has weaknesses, as discussed below, this approach can be used successfully provided the cognitive factors involved in the compared tasks are considered carefully.

Using Factorial and Parametric Designs to Overcome Limits in the Subtractive Approach

The subtractive method may be used to finely dissect a particular cognitive function, but it may be inadequate due because it falsely assumes that the neural correlates of cognitive functions add by *pure insertion*. In a serial subtractive design, task conditions are designed in which cognitive components are added one by one. Under the assumption pure insertion, differences between each task condition will reflect processing of the new cognitive component. However, if pure insertion is not true, the addition of a new cognitive component may alter the way the brain handles the existing components. Because there may be *interaction* between cognitive components, *factorial analysis* may be used to account for this interaction (Friston et al., 1996). This approach was illustrated by Friston et al. (1996) by investigating the neural correlates of object recognition and phonological retrieval in an object naming task. The authors tested the hypothesis that the inferior temporal activation during object naming reflected phonological retrieval. They did so using both a serial subtractive method and using a factorial method. The serial subtraction experiment involved three conditions: task A, saying “yes” in

response to a colored shape, task B, saying "yes" in response to a colored object, and task C, naming a colored object. Task A involved visual analysis and speech, task B involved visual analysis, speech, and object recognition, and task C involved visual analysis, speech, object recognition, and phonological retrieval. The responses to these task conditions were examined using PET. The inferior temporal region was activated when subtracting A from B, but not when subtracting B from C. This implied that this region was involved in object recognition, but not phonological retrieval. In the factorial design, a task D was added: naming the color of a colored shape. Task D involved visual analysis, speech, and phonological retrieval, but *not* object recognition. The PET responses to these four task conditions were analyzed using a two-way ANOVA, allowing the detection of a main effect of object recognition (B & C vs. A & D), a main effect of phonological retrieval (C & D vs. A & B), and an interaction between the two. The results showed that at the inferior temporal lobe, there was both a main effect of phonological retrieval and an interaction between phonological retrieval and object recognition. That is, the response to object recognition varied with the addition of phonological retrieval, and vice versa. The interaction demonstrated that for this experiment, the assumption of pure insertion was not true by revealing a response in the inferior temporal lobe to object recognition that was missed using the subtractive approach. Thus factorial designs can be used to examine separately the neural correlates of cognitive components that may interact.

Like factorial designs, parametric designs attempt to overcome the problem with cognitive subtraction. They do so by comparing the MR signal to a task parameter with multiple values. This allows the identification of brain regions whose activity covaries with the task-related parameter, rather than regions that are merely more active during the task. For example, one experiment regions whose responses covaried with word presentation rate (Buchel et al., 1998).

Whereas all words activated bilateral frontal regions irrespective of rate, presentation rate predicted responses in bilateral occipitotemporal regions. This shows that investigating covariations of fMRI responses with experimental parameters may finely dissect neural correlates of one task parameter. In this case, the parametric approach distinguished set-related activity during word reading from individual stimulus perception. Parametric analysis may also be used in experiments involving complex stimuli, such as photographs, to separate the effects of interest from other confounds present in the complex stimulus. This approach was applied in this dissertation using emotional ratings of photographs as a parameter in analyzing fMRI data.

CHAPTER 3
PRELIMINARY DATA: DISSECTING THE NEURAL CORRELATES OF DISGUST

This experiment investigated the ability of fMRI to distinguish nuances in the negative emotional content of picture stimuli. Previous studies showed that the amygdala and occipito-temporal cortex (OTC) respond to pictures rated as emotionally arousing. Responses in the amygdala to fearful faces have been dissociated from responses in the insula to disgusted faces. It is not clear whether the amygdala/OTC and insula respond selectively to arousing and disgusting pictures. In this fMRI study, healthy volunteers viewed pictures of contamination, human mutilation, threat, and neutral scenes during scanning, and then rated pictures for the "basic" emotions, including disgust. The anterior insula responded to contamination and mutilation but not threat, while the OTC responded to threat and mutilations more strongly than contamination. The above activations were predicted by disgust and arousal ratings respectively. Additionally, mutilations uniquely activated the right superior parietal cortex. No response was detected at the amygdala. These results support selective disgust processing at the insula, and suggest distinct neural responses to contamination and mutilation. The use of fMRI in investigating components of emotion processing is feasible, but subsequent studies should improve imaging of the amygdala.

Introduction

The role of the insula in affective processing has been the topic of recent debate. Case reports from patients with insula lesions and Huntington's disease describe impaired recognition of facial expressions of disgust, and in some cases, impaired ability to feel the emotion of disgust itself (Adolphs et al., 2003;Calder et al., 2000;Sprengelmeyer et al., 1996). In healthy volunteers, functional brain imaging experiments using facial expression recognition tasks have indicated selective activation of the insula to facial expressions of disgust (Phillips et al.,

1997;Sprengelmeyer et al., 1998). However, results from experiments using pictures such as body products to induce disgust, question the notion of selective disgust processing at the insula. Although an insular response to disgust-inducing pictures was reported in a study of obsessive-compulsive disorder (OCD) patients (Phillips et al., 2000), two subsequent studies of healthy controls found equal activation of the insula to both disgust- and fear-inducing pictures (Schienle et al., 2002;Stark et al., 2003). Surprisingly, disgust-inducing pictures activated the amygdala more than fear-inducing pictures in these studies; the authors explain that their disgust-inducing pictures elicited high disgust ratings, while their fear-inducing pictures elicited only moderate fear ratings. A recent study in our lab extended the previous OCD study (Phillips et al., 2000) by adding fear-inducing pictures (Shapira et al., 2003). In this case, we found greater insula activation for disgust-inducing pictures than fear-inducing pictures in both healthy volunteers and patients with OCD. Considering the discrepancy between our results and those of the studies above (Schienle et al., 2002;Stark et al., 2003), we decided to examine potential differences in study methodologies. The former studies used pictures of contamination (e.g. spoiled food and body products) and mutilations (e.g. injuries and corpses) to induce disgust, whereas ours used only pictures of contamination. We therefore designed a study to test separately the effects of these two types of pictures.

The insula has been associated with a range of functions, including visceral and gustatory processing (Wicker et al., 2003), autonomic regulation (Critchley et al., 2003), and self-generated affective experiences (Phan et al., 2002); thus a general affective or disgust-specific role for the insula are both plausible. Schienle et al. (2002) suggest that a shared affective pathway is sufficient to explain the insular response to affective pictures. The current study re-

examines this conclusion by presenting pictures of contamination and mutilation separately to test whether the insular response is specific to food-related disgust.

We hypothesize first that the insula responds selectively to disgust-inducing pictures, and second that there is a distinct neural response to pictures of contamination and pictures of mutilation. The first hypothesis predicts that pictures of contamination or mutilation or both will cause greater activation at the insula than fear-inducing pictures. The present study therefore uses functional magnetic resonance imaging (fMRI) to compare these two types of disgust-inducing pictures with fear-inducing and neutral pictures, in order to assess the validity of combining pictures of mutilation and contamination in a single category. In addition to performing standard exploratory analysis using statistical activation maps, we examine the neural response to the affective pictures in detail using signal time-courses from selected regions of interest (ROIs).

Methods

Subjects

Eight healthy volunteers (4 male) aged 20-26 gave written informed consent in accordance with a protocol approved by the Institutional Review Board at the University of Florida. According to self-report, 7 were right-handed, and 1 was left-handed. The volunteers denied taking any psychiatric medication at the time of the scan and gave no history of psychiatric or neurological disorders.

Disgust Picture Paradigm

Pictures were selected from the International Affective Picture System (IAPS) (Center for the Study of Emotion and Attention [CSEA-NIMH], 2001) and were divided into four categories: “contamination”, “mutilation”, “threat” and “neutral” which were defined as follows: contamination pictures depicted of scenes associated with poor hygiene or poisons (e.g. spoiled food, bodily waste, garbage, pollution); mutilation pictures showed human injuries or disease

(e.g. murder victims, traffic accidents, tumors, birth defects); threat pictures showed imminent attacks (e.g. humans with guns or knives, dogs, snakes); and neutral pictures depicted various scenes with low arousal and medium pleasure ratings (Lang et al., 2001) (e.g. landscapes, household tools, non-threatening animals).

The stimuli were presented using an Integrated Functional Imaging System (IFIS, MRI Devices, Inc., Waukesha, WI) with a 7" LCD screen at 640 X 480 pixel resolution, mounted over the subject's head and viewed using a fixed mirror. The screen subtended approximately 14° x 11° of the visual field. A PC running E-Prime (Psychology Software Tools, Pittsburgh, PA) began presenting each task in synchronization with the first RF pulse of each scan. Each emotion category was presented during a separate MRI scan in order to avoid the fatigue or boredom that may result from viewing a single, long sequence. The order of the runs was randomized for each participant. Each run consisted of six alternating emotional and neutral picture blocks (21 sec long), interspersed with 9-second fixation blocks. Each block contained 14 pictures selected randomly (without replacement) from the list for that category, and each picture was presented for one second, followed by 0.5 seconds of fixation. Participants were instructed to view the pictures passively, keep their eyes open, and to avoid repressing or exaggerating their emotional response.

After scanning, each participant rated 15 randomly chosen pictures on a scale from 1 to 5 (5 being the most intense emotion) for each of the following "basic" emotions: happiness, sadness, fear, anger, disgust and surprise (Ekman, and Friesen, 1976). Dimensional ratings were taken from the normative set provided with the IAPS. These were ratings from 1 to 9 for pleasure, arousal, and dominance, with 9 indicating the viewer felt most pleasant, most aroused,

and most dominant respectively. Finally, each participant completed a 32-item questionnaire designed to indicate their sensitivity to disgust (Haidt et al., 1994).

Functional Imaging Data Acquisition

MR images were acquired using a 3 Tesla Allegra system (Siemens, Munich, Germany). Anatomical imaging used a standard MPRAGE sequence with a 240mm square field of view at 256 × 256 pixel resolution in the axial plane, and 160 slices of 1.0-1.4mm thickness. Functional data were gathered using echo-planar imaging (EPI) sensitive to blood oxygen level-dependant (BOLD) signal (TR = 3000ms, TE = 30ms, flip angle = 90°, FOV = 240mm, matrix = 64 × 64). Twenty-four slices were collected in the axial plane with a 6 mm thickness and 0 mm gap. Each functional run lasted 3 min 9 sec and consisted of 63 volumes, the first two of which were discarded before analysis due to their T1 saturation.

Functional Imaging Data Analysis

Data were analyzed using BrainVoyager 2000 v. 4.9.6 (Brain Innovations, Maastricht, Holland). Each anatomic scan was normalized to Talairach space, and the transformation parameters saved. The in-plane functional images from each participant were then co-registered with the pre-transformation anatomic scan, and converted into a 3D volume time-course in Talairach space using the saved transformation parameters. Finally, the 3D functional data underwent 3D motion correction and linear trend removal.

Voxel-wise statistical activation maps were generated using a general linear model (GLM) in which the predictors were an estimated hemodynamic response to each emotional condition. Contrasts between predictors were used to calculate the relative contribution of each predictor to the variance in the BOLD signal. Unless otherwise stated, the statistical threshold was set to $p <$

0.05 with Bonferroni correction for multiple comparisons, and the minimum cluster size was 100 mm^3.

Region of interest (ROI) analyses were performed within selected clusters of significantly activated voxels. Within each ROI, the BOLD responses for each condition were visualized using time-locked averaging of the percentage signal change relative to fixation. A GLM was calculated for the mean signal from the ROI, and the modeled amplitude of each predictor (the beta weight) was used to describe the size of the hemodynamic response. Unlike the statistical activation value, which reflects how well the model fits the data, the beta weight describes the BOLD response, which is assumed to be proportional to neural activation (Ogawa et al., 1992).

Results

Emotion Ratings

All three emotional conditions were rated as evoking significantly less pleasure, more arousal, and more dominance than neutral according to the mean IAPS scores for each picture set (Lang et al., 2001) (Tables 3-1 & 3-2). Mutilation was less pleasant than contamination and threat, and contamination was less arousing than threat and mutilation. According to our own subjects' ratings of "basic" emotions, the threat condition elicited more fear than the other three conditions and the contamination and mutilation conditions each elicited more disgust than threat and neutral. The mutilation condition also elicited more sadness than neutral. (For all the above comparisons $p < 0.001$, corrected for multiple comparisons.) The mean ± standard deviation disgust sensitivity score was 13.4 ± 4.0 (males: 13.3 ± 3.4, females: 13.6 ± 5.2). The mean for American adults is approximately 16 (males: 14, females: 18) (Haidt et al., 1994).

fMRI Data

Exploratory statistical activation maps were generated by contrasting each emotional condition with neutral using the GLM (see Methods: data analysis). Figure 3-1 illustrates clusters

of activation seen in the anterior insula and occipito-temporal cortex (OTC). See Tables 3-3, 3-4, & 3-5 for a full list of activated regions.

The anterior insula was activated bilaterally in both the contamination and mutilation conditions. No significant activation was found in the insula for the threat condition at the threshold $p < 0.05$ corrected. The extent of activation in the OTC increased in from contamination to threat to mutilation respectively. Activation for mutilation extended into the midline occipital cortex and posterior cingulate and was additionally seen in the thalamus, ventral striatum, superior parietal cortex and several prefrontal regions (Table 3-5). No significant signal changes were found at the amygdala, but detailed examination of the EPI (functional) images revealed loss of signal at the amygdala due to susceptibility artifact.

Comparisons between emotions revealed no unique activation for contamination or threat, but mutilation condition activated the right superior parietal cortex. The contrasts (contamination - threat) and (mutilation - threat) each showed activation in the left anterior insula at a threshold of $p < 0.0001$ uncorrected, but this did not achieve the stricter threshold of $p < 0.05$ corrected. Clusters of activation for ROI analysis were selected from those contrasts showing significant differences between emotional conditions; thus the insula ROI was derived from the contrast [(contamination + mutilation) - neutral] and the OTC ROI from the contrast [(mutilation + threat) - neutral]. The left and right ROIs were combined for analysis. The right superior parietal ROI was selected from the contrast [mutilation - (contamination + threat)]. All three ROIs are illustrated in Figure 3-2.

Time-locked averaging of the BOLD signal across conditions (see Figure 3-3 A-C) showed a phasic response to all picture conditions (including neutral) in the OTC. This response was enhanced in the emotional conditions: the enhancement was smallest for contamination, greater

for threat, then greatest for mutilation. At the insula, viewing neutral pictures evoked no change in signal, but contamination and mutilation again caused a phasic increase. Viewing threat pictures caused a small response, although this failed to reach the threshold for statistical significance during the exploratory analysis (Figure 3-1). Signal in the right superior parietal cortex increased in response to mutilation pictures, but was indistinguishable from neutral during the other conditions.

The widespread activation for mutilation pictures (see Figure 3-1) may reflect the high affective arousal ratings for these pictures, particularly the amplitude of the signal increases in the OTC; also, activity in the anterior insula suggested a relationship with the disgust rating. We therefore tested the correlations between picture ratings and BOLD signal change, represented by beta weight (see Methods: Data analysis). Since the experimental design did not include comprehensive picture ratings for each subject, the ratings were pooled across subjects. Arousal rating predicted signal change in the OTC [$r^2 = 0.98$, $p < 0.05$], and disgust rating marginally predicted signal change in the anterior insula [$r^2 = 0.85$, $p = 0.08$] (see Figure 3-3 D+E). The complementary correlations were not significant: disgust rating with OTC signal change [$r^2 = 0.61$, $p = 0.22$] and arousal rating with insular signal change [$r^2 = 0.28$, $p = 0.47$]. OTC signal change was also predicted by ratings for happiness, pleasure and dominance, but these were each correlated with the arousal rating [respectively, $r^2 = 0.97$, 0.85 & 0.999, $p < 0.05$, $p = 0.08$ & $p = 0.0005$], suggesting that, in this case, these ratings are confounded with a common factor. The disgust rating did not correlate significantly with any other ratings. Each subject's disgust sensitivity score was compared with that individual's signal change in the OTC and insula for each emotional condition, but no significant correlations were found.

Discussion

The aim of this study was to compare the neural responses to two potentially different types of disgust. Contrary to previous studies comparing disgust- and fear-inducing pictures, (Schienle et al., 2002;Stark et al., 2003) we found that disgust significantly activated the insula while fear did not. Furthermore, we showed that the insular response correlated with feelings of disgust, but not with feelings of arousal. Secondly, we showed distinct neural responses to viewing pictures of contamination and mutilation. Specifically, viewing pictures of mutilation caused greater activation of the OTC, and unique activation of the right superior parietal cortex.

The data presented here are insufficient to explain the failure of two previous studies (Schienle et al., 2002;Stark et al., 2003) to find a specific insular response to disgust in terms of the effect of combining pictures of contamination and mutilation, since the insula responded to both conditions. It is possible that our small (and statistically non-significant) insular response to threat was because our pictures evoked less fear than those of the other two studies. A comprehensive comparison of picture ratings between studies is not possible here but the fear ratings for our threat picture set (2.7 out of 5, equivalent to 4.8 out of 9) are close to those of Schienle et al. and Stark et al. (5.5 and 4.8 out of 9 respectively). Furthermore, if we are to accept the interpretation that activity in the insula reflects a shared affective system, then our study should have shown greater activity in the insula to threat than to contamination, since the threat pictures were rated as more arousing and less pleasant than contamination. One possible explanation is that 1.5 Tesla MRI (used in the previous studies) is not sufficiently sensitive to BOLD effects to detect the relatively small differences between the fear and disgust responses at the insula that are detectable at 3 Tesla (used in the current study).

Previous studies have also suggested that activation of the OTC is influenced by emotional intensity (Lang et al., 1998;Schienle et al., 2002;Shapira et al., 2003;Stark et al., 2003). These

visual areas do not encode emotion, but receive feedback from emotion-processing regions such as the amygdala (Rolls, 1999). Thus, although we failed to image the amygdala in this study, enhancement of ventral visual processing may be thought of as a proxy for amygdala activity (Sabatinelli et al., 2005). The most compelling evidence we found for a specific response to disgust in the insula is found in the correlations between disgust rating and insular response, and arousal rating and occipito-temporal response (Figure 3-3 D+E). These suggest a double dissociation between the insula, processing information related to disgust, and the OTC, processing general affective arousal. These findings are compatible with the existence of a common affective pathway, but suggest that this simple model is insufficient to explain activity at the insula. Activation of the insula by both mutilation and contamination pictures suggests that the insular response to disgust is more related to the emotional feeling of disgust rather than the gustatory content of the eliciting stimulus. An electrical recording study in humans provides support for a late (300 ms) response to emotional stimuli at the insula, likely reflecting a conscious feeling rather than earlier processing of gustatory content (Krolak-Salmon et al., 2003).

We recognize several shortcomings of this study. Since we were unable to image the amygdala, we had to use occipito-temporal activation as a proxy for the amygdala response. Although in this study, activity in the insula was not correlated with affective arousal, the insula influences autonomic arousal (Critchley et al., 2003), and we cannot rule out the insula's influence on occipito-temporal activity. The affective ratings of our picture sets may be confounded with other features unique to each set, such as the lack of human faces in the contamination set, or the abundance of the color red in the mutilation set. Future studies should use imaging parameters able to image the amygdala, take physiological measures of arousal

(such as heart rate and skin conductance) and specifically account for possible confounds during selection of picture sets. (It should be noted that not all studies report confirmation of proper amygdala imaging, and that artifact is common at higher magnetic fields, i.e. 3 Tesla (Merboldt et al., 2001).)

The unique activation of the right superior parietal cortex by mutilation pictures is an interesting, new finding that should be further explored by future studies. A previous case study proposed a parietal pathway for processing acted-out emotions (Adolphs et al., 2003). This pathway may be more responsive to mutilation pictures if the viewer processes them by mentally re-enacting the bodily condition of the victim in the picture. This view is further supported by studies locating mirror neurons for bodily actions in the parietal cortex (Buccino et al., 2004). Whether mutilation pictures evoke a distinct emotion, for example "horror", is an interesting question for further study. It has been suggested that horror is a blend of disgust and fear, and it is interesting to note that mutilation may be viewed for pleasure in art or entertainment (McNally, 2002).

In conclusion, our findings suggest that the OTC and the insula process different affective information, as reflected by arousal and disgust ratings respectively. Modulation of occipito-temporal activity by feelings of arousal is well modeled by the concept of a shared affective network processing basic affective dimensions. However, the apparently disgust-specific activity in the insula supports the idea that emotional categories may have distinct neural representations. We also suggest that future studies consider contamination and mutilation pictures separately. Whether mutilation pictures evoke a distinct emotion (perhaps "horror") is a question best answered by future research.

Table 3-1 Affective ratings (dimensional)

Picture set	Pleasure	Arousal	Dominance
Contamination	3.2 ± 0.8	4.9 ± 0.8	4.7 ± 0.6
Mutilation	1.9 ± 0.6	6.3 ± 0.7	3.3 ± 0.6
Threat	3.0 ± 0.8	6.3 ± 0.9	3.3 ± 0.7
Neutral	5.6 ± 0.9	3.4 ± 1.0	6.0 ± 0.6

Pleasure, arousal and dominance ratings are out of nine, and were taken from the IAPS data.

Table 3-2 Affective ratings (categorical)

Picture set	Happiness	Sadness	Fear	Anger	Disgust	Surprise
Contamination	1.2 ± 0.7	1.3 ± 0.6	1.4 ± 0.8	1.1 ± 0.3	2.6 ± 1.0	1.3 ± 0.5
Mutilation	1.0 ± 0.0	2.4 ± 1.3	1.6 ± 0.9	1.4 ± 0.6	3.2 ± 1.3	1.6 ± 0.9
Threat	1.0 ± 0.0	1.3 ± 0.5	2.7 ± 1.0	1.6 ± 1.0	1.5 ± 1.0	2.0 ± 1.3
Neutral	1.6 ± 0.9	1.0 ± 0.0	1.1 ± 0.2	1.0 ± 0.0	1.1 ± 0.2	1.2 ± 0.4

Happiness, sadness, fear, anger, disgust, and surprise ratings are out of five, and were obtained from subjects in the current study.

Table 3-3 Clusters of activation for (threat - neutral)

Region	Side	BA	X	Y	Z	Size	t(478)
OTC	R	37	46	-62	-4	4314	8.7
OTC	L	37	-42	-64	-3	2855	8.0
Parahippocampal gyrus	L	36	-26	-39	-3	219	-6.6

Only clusters >100 voxels shown. L: left, R: right. BA: Brodmann's Area. X, Y and Z refer to Talairach co-ordinates (mm). Size: number of 1mm3 voxels. t: mixed effects statistical score (degrees of freedom). Negative t score denotes decrease relative to neutral. OTC: occipito-temporal cortex.

Table 3-4 Clusters of activation for (contamination - neutral)

Region	Side	BA	X	Y	Z	Size	t(478)
Insula	R	13	31	20	2	571	6.6
Insula / frontal operculum	L	13, 47	-38	27	0	1650	7.7
Middle frontal gyrus	R	46	44	17	23	119	6.2
OTC	L	37	-45	-55	-8	1909	7.4
OTC	R	37	45	-49	-10	554	6.7

Only clusters >100 voxels shown. L: left, R: right. BA: Brodmann's Area. X, Y and Z refer to Talairach co-ordinates (mm). Size: number of $1mm^3$ voxels. t: mixed effects statistical score (degrees of freedom). OTC: occipito-temporal cortex.

Table 3-5 Clusters of activation for (mutilation - neutral)

Region	Side	BA	X	Y	Z	Size	t(478)
Cerebellum	B		1	-72	-24	136	6.2
Insula	L	13	-33	22	1	526	6.5
Insula / frontal operculum	R	13, 47	39	22	-1	1614	7.4
Medial frontal gyrus	R	8	5	40	36	248	6.6
Middle frontal gyrus	R	10, 46	33	46	13	134	-6.1
Midline occipital		17-19, 29-31	0	-67	9	6818	8.2
OTC	L	37	-38	-61	-10	8419	10.0
OTC	R	37	42	-60	-7	14555	11.3
Parahippocampal gyrus	R	36	19	-52	0	290	6.1
Parahippocampal gyrus	L	36	-19	-52	-3	844	7.4
Precentral gyrus	R	6	45	-3	35	481	6.3
Superior frontal gyrus †	B	9	-4	57	30	2165	8.7
Superior frontal gyrus	L	8	-11	24	57	144	6.6
Cuneus	L	19	-8	-89	33	279	6.4
Cuneus & precuneus	R	19	25	-69	39	8924	9.6
Cuneus & precuneus	L	19	-23	-76	32	1132	6.4
Intra-parietal sulcus	L	7	-30	-55	38	151	6.1
Thalamus	L		-6	-15	13	109	6.0
Ventral striatum	R		21	0	-4	108	6.6

Only clusters >100 voxels shown. L: left, R: right, B: bilateral. BA: Brodmann's Area. X, Y and Z refer to Talairach co-ordinates (mm). Size: number of $1mm^3$ voxels. t: mixed effects statistical score (degrees of freedom). Negative t score denotes decrease relative to neutral. OTC: occipito-temporal cortex. †: this cluster may not reflect neural activity because it is partly outside the brain and its shape corresponds to the anterior sagittal sinus.

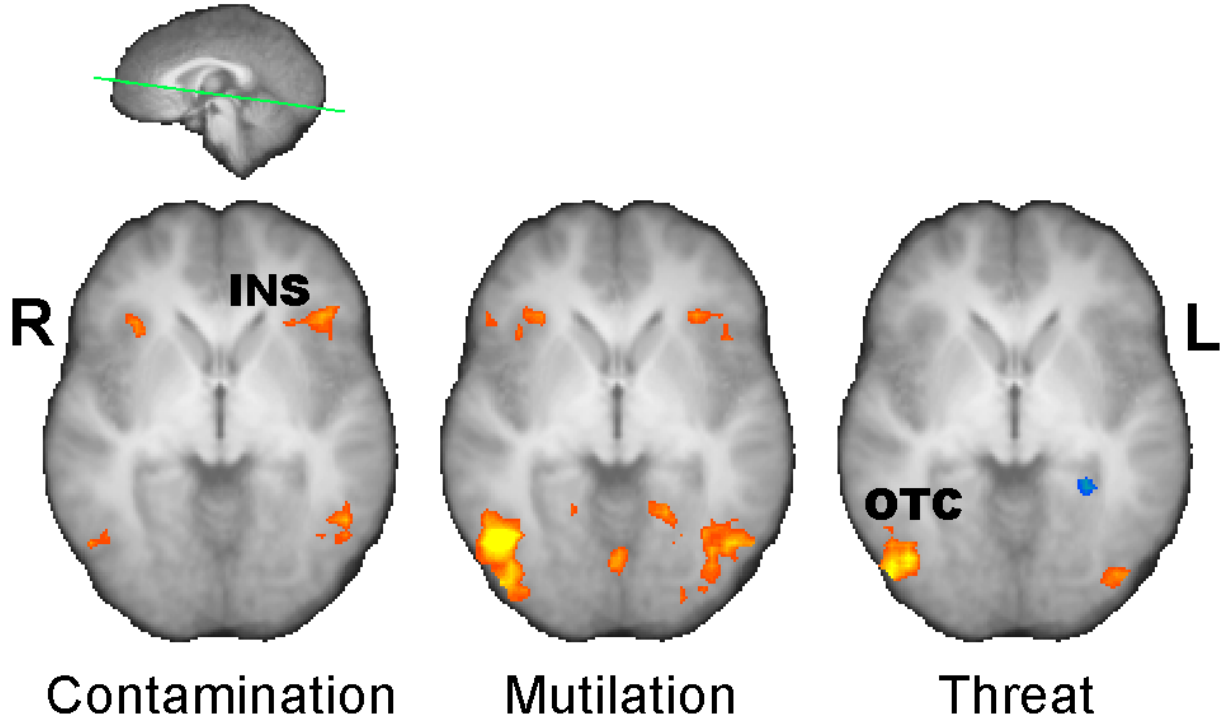

Figure 3-1. Statistical maps showing contrasts between each emotional condition and neutral. Contamination and mutilation activated the anterior insula. The occipito-temporal cortex (OTC) responded to all three emotional conditions, but comparatively weakly to contamination. Red / yellow: emotion > neutral, blue: neutral > emotion. Green line in inset shows slice angle (8º from ACPC). Threshold: $p < 0.05$, corrected for multiple comparisons, minimum cluster size: 100 mm^3.

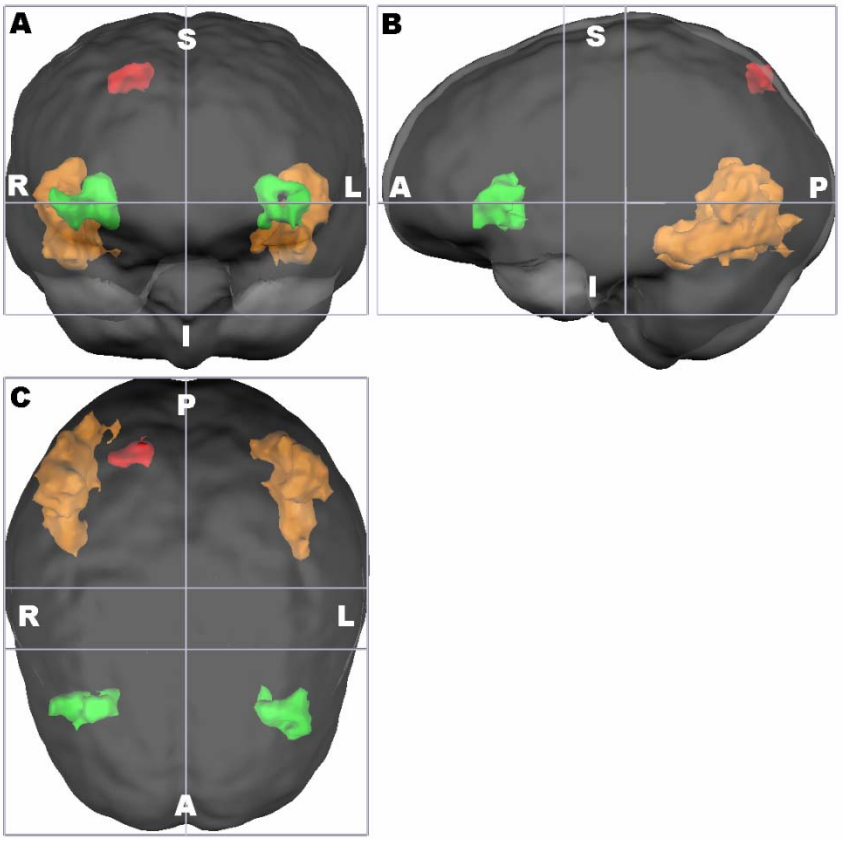

Figure 3-2. "Glass brain" view of regions of interest. Green: insula, orange: occipito-temporal cortex, red: right superior parietal cortex. A: view from front, B: view from left, C: view from top. The contrasts from which these ROIs were selected are described in Methods: Functional imaging data analysis. Internal axes denote the anterior and posterior commissures. L: left, R: right, A: anterior, P: posterior, S: superior, I: inferior.

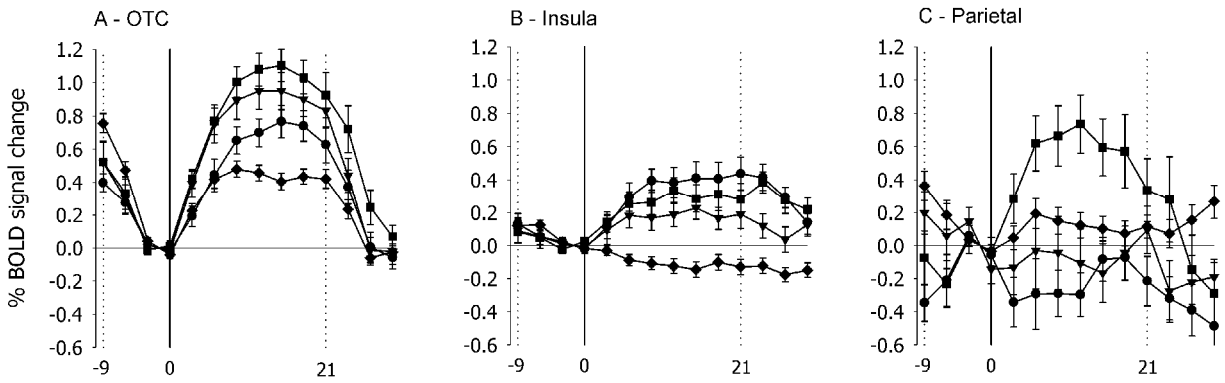

Figure 3-3. BOLD responses. A) There is an occipitotemporal response to all conditions, but this is enhanced in the emotional conditions. B) The insula did not respond to neutral pictures, but showed the greatest response to contamination pictures. The small response to threat pictures did not reach significance in the exploratory analysis (Figure 1). C) The superior parietal ROI responded only to mutilation pictures. ●: contamination, ■: mutilation, ▼: threat, ◆: neutral. Solid vertical lines indicate start of picture block, dotted vertical lines indicate start of fixation block.

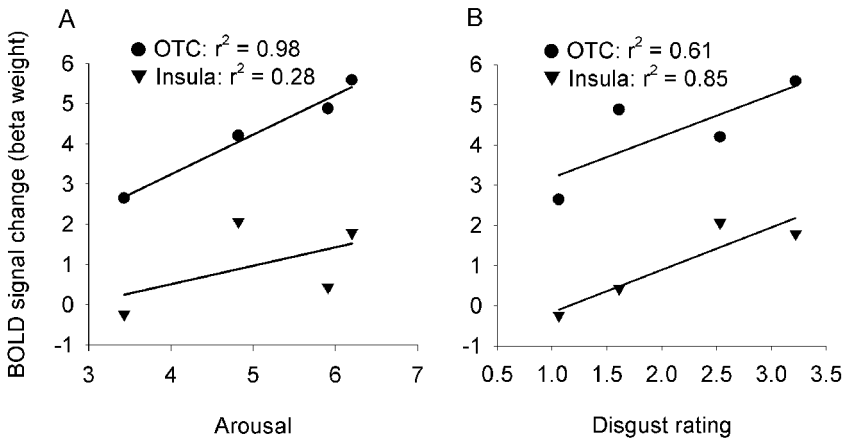

Figure 3-4. Correlations with emotion ratings. For each picture set, BOLD response amplitude (Beta weight) is plotted against A) arousal rating & B) disgust rating. Arousal predicted ventral visual activity, whereas disgust predicted activity in the anterior insula. ●: occipito-temporal cortex, ▼: anterior insula.

CHAPTER 4
FACE MATCHING AND THE AMYGDALA: BOTTOM-UP EMOTION PROCESSING OR NOT?

Previous studies have investigated top-down modulation of bottom-up emotion processing in the amygdala using a face matching and labeling task. The face matching component of this task has been shown to activate the amygdala reliably, and is thought to elicit bottom-up processing of the facial features that communicate emotion. However, facial feature matching may also elicit intentional, knowledge-based processing in pursuit of task demands, or top-down processing. Therefore, in order to distinguish facial feature matching from emotion processing, and presumably thereby to dissect an emotional response at the amygdala, we modified the face matching task to include an intermediate control condition in which neutral faces were matched by identity. The left and right amygdalae responded to both the emotion and the identity matching conditions, and the only selective response to emotion matching was at the left inferior prefrontal sulcus. The left amygdala response habituated to emotion matching but not to identity matching. Although the amygdala has been described as a "fear module", a growing body of work suggests that it is a more general "relevance detector". We concluded that the amygdalar response to face matching is driven at least in part by relevance detection, independent of emotion processing, although there appears to be additional emotion-specific processing at the left amygdala. These results suggest that the face matching task is not a valid paradigm to investigate bottom-up processing of facial emotion in the amygdala.

Introduction

The amygdala has been described as an emotion processing module specialized for fear, enabling both the experience of fear, and the recognition of fear in others. While this notion is supported by lesion studies (Adolphs et al., 1994) and functional neuroimaging (Morris et al., 1996), other data suggest that the amygdala has a more general role, described as relevance

detection (Sander et al., 2003). For example, the amygdala also responds to disgusting scenes (Schienle et al., 2002), abstract figures associated with food reward (Gottfried et al., 2003), neutral faces of a different race (Hart et al., 2000), and novel neutral faces (compared with previously-viewed neutral faces) (Schwartz et al., 2003).

The amygdalar response to facial expressions of emotion is gaining clinical relevance as a paradigm for studying anxiety and affective disorders. An increased response to fearful or sad faces has been shown in patients with depression (Surguladze et al., 2005) as well as post-traumatic stress disorder (Rauch et al., 2000;Shin et al., 2005). Furthermore, in depression, this hyperresponsivity has been reversed by drug treatment (Fu et al., 2004;Sheline et al., 2001). Several of the above investigators have suggested a correlation between altered amygdala activity and previous behavioral data showing impaired facial expression recognition in patients with depression (Gur et al., 1992). These studies used a variety of tasks, and often did not control for non-emotional face processing. A more integrated picture of the neurobehavioral alterations surrounding amygdalar dysfunction could be obtained using a well-established task to dissociate emotional and non-emotional face processing.

Several groups have reported a robust amygdala response to a task that requires participants to match faces by emotional expression (Hariri et al., 2000;Hariri et al., 2002c;Paulus et al., 2005;Piggot et al., 2004;Wang et al., 2004). Matching facial expressions requires explicit evaluation of emotion, but without the explicit use of verbal labels, which may inhibit the amygdala response by activating the ventral prefrontal cortex (Hariri et al., 2000).

In order to clarify whether the amygdalar response to matching emotional faces is specifically due to the perception of emotion, we designed a variant of the matching task from Hariri et al. (2000). We added an intermediate control condition – matching neutral faces by

identity – to dissect more precisely the emotional component of the matching task. If the amygdalar response specifically reflects emotion processing, then it should appear only in the emotion matching condition. Other regions involved in the perception of faces, such as the fusiform face area (Kanwisher et al., 1997) and the superior temporal sulcus (Chao et al., 1999), should respond to both emotion matching and identity matching.

Methods

Subjects

Twelve healthy participants (six female), aged 18 – 53 (mean 29) years, were recruited as approved by the University of Florida's Institutional Review Board. All participants were right-handed and had normal or corrected-to-normal vision. None reported any neurological or psychiatric history, nor use of psychoactive medications for the previous six months.

Face Matching Task

The matching task consisted of three conditions: emotion, identity, and control. In each condition, participants were shown a target face above two probe faces, and then had to choose which probe matched the target (Figure 4-1). In the emotion condition, participants were asked to match the faces by their expressed emotion (happiness, fear, or anger). In the identity condition, participants were asked to match neutral faces by identity. In the control condition, participants were asked to match the pixilated patterns derived from neutral face pictures; thus all three conditions presented objects with the same dimensions and shades of gray. The task was ordered in blocks of six 3-second trials of the same condition, preceded by a 3-second instruction screen. The block condition was varied in a fixed sequence that repeated four times and was counterbalanced across participants (emotion > identity > control or control > identity > emotion). The entire run consisted of twelve 21-second tasks blocks interspersed with thirteen 9-second rest blocks and lasted 3 min 9 sec. During rest, a fixation cross was displayed. A total of

48 grayscale face portraits were presented from the series "Pictures of Facial Affect" (Ekman, and Friesen, 1976), with six actors of each gender posing happy, fearful, angry, and neutral expressions. Twelve control patterns were created by shrinking neutral face pictures to 8 x 12 resolution, randomizing the pixels, and enlarging to original size. Within trials, probe and target faces were the same gender, and an equal number of trials of each gender were presented in each block. Each actor's face appeared an equal number of times during the experiment. In the emotion condition, one actor was selected for the probe face, and a second actor for both of the target faces. The pictures subtended approximately 3.6° x 5.4° (target) and 2.9° x 4.3° (probes) of the visual field (the target was larger to help distinguish it from the probes). Participants selected the left or right target by pressing a button under their index or middle finger respectively, causing the selected target to be outlined in yellow. The participants practiced each condition inside the scanner before the experimental run until they felt confident performing the task.

The stimuli were presented using an Integrated Functional Imaging System (IFIS, MRI Devices, Inc., Waukesha, WI) with a 7" LCD screen at 640 X 480 pixel resolution, mounted over the subject's head and viewed using a fixed mirror. The screen subtended approximately 14° x 11° of the visual field. A PC running E-Prime (Psychology Software Tools, Pittsburgh, PA) began presenting each task in synchronization with the first RF pulse of each scan. Responses were collected with a MRI-compatible button glove attached to the participant's right hand.

Functional Imaging Data Acquisition

Brain images were acquired using a Siemens Allegra 3 Tesla scanner (Siemens, Munich, Germany) with a standard head coil. Anatomic images were acquired using an MPRAGE sequence in the sagittal plane at 1.0 mm^3 resolution, TR = 1780ms, TE = 4.38ms, flip angle = 8°. Functional images were acquired using a gradient echo planar imaging (EPI) sequence sensitive

to blood oxygen level-dependant (BOLD) contrast in the axial orientation (parallel to the AC-PC line), covering the whole brain with 36 slices, 3.8mm thick (0mm gap) with a 240mm field of view and a matrix size of 64 x 64 voxels (in-plane resolution = 3.75mm), TR = 3000ms, TE = 30ms, flip angle = 90°. A total of 125 brain volumes were acquired (3min 15sec scan time) and the first two volumes were discarded before analysis to allow for T1 equilibration.

Functional Imaging Data Analysis

MR data were analyzed using BrainVoyager 2000 (v. 4.9.6, Brain Innovations, Maastricht, Holland). The functional images were coregistered with anatomic images, and normalized to Talairach space for each participant. Functional data underwent 3D motion correction, linear trend removal and slice scan time correction (the slice data in each volume were time-shifted to the start of the TR by interpolation). High- and low- frequency noise was removed using low- and high- pass filters with cut-off frequencies of 10/123 Hz and 1/123 Hz respectively. Spatial smoothing was applied using a Gaussian filter of 5.7mm full-width half maximum.

Regions of task-related brain activity were estimated using general linear modeling. A reference function, or predictor, was created for each condition by convolving the block presentation time course with an estimated hemodynamic response function (Boynton et al., 1996). The signal at each voxel was modeled with a weighted combination of the three predictors using least squares fitting. Statistical maps were created using random effects analysis. This conservative approach looks for consistent differences between predictors' weighting across participants, preventing data from one or two participants from dominating the analysis. Clusters of voxels with significant differences between predictors were selected by setting a statistical threshold of $t(11) > 4.0$ ($p < 0.002$ uncorrected) and a minimum cluster size of 100 mm^3 (except at the amygdala - our *a priori* region of interest - where smaller clusters were allowed).

For each cluster of significant voxels, we validated the GLM results by plotting the mean BOLD response to each condition, as previously described (Wright et al., 2004). The percentage signal change was calculated for each block relative to the preceding resting signal, and then averaged across blocks and participants for each condition. In order to investigate habituation, the BOLD response to each condition was calculated separately for each of the four repetitions of that block, averaged across participants. The mean peak BOLD response was calculated from the period of peak activity at 9 – 18 seconds after block onset. The magnitude and significance of any modulation of mean amplitude over time were calculated using linear regression.

To confirm that the MRI parameters used were able to detect signal at the amygdala, we visually inspected the functional images using an outline of the amygdala drawn from the average anatomic image according to the guidelines of Brierley et al. (Brierley et al., 2002). In two out of the twelve participants, susceptibility artifact from the nasal sinuses obscured the amygdala. Because amygdala activation could be detected with or without these participants, they were included in the analysis to maximize statistical power in the rest of the brain.

Results

Behavioral Data

Participants' responses were significantly faster and more accurate in the identity condition, compared with emotion and control (Table 4-1). On debriefing most participants reported that the identity condition was the easiest of the three.

fMRI Data

Significant differences in modeled signal amplitude (“activations”) are summarized in Tables 4-2 & 4-3. Emotion-specific activations found using the conjunction of the contrasts (emotion - identity) and (emotion - control) occurred at the left inferior frontal sulcus (Figure 4-2) and right precentral gyrus (not shown). The BOLD response at the inferior frontal

sulcus appeared to be specific to the emotion condition (Figure 4-2A), while at the precentral gyrus, the magnitude of the response to identity appeared to be intermediate between that of control and emotion. Emotion-specific deactivations were found at the left transverse temporal sulcus and the pregenual anterior cingulate gyrus (Figure 4-4B). Specifically, the BOLD response at the temporal region was negative during emotion matching but remained near baseline levels during the identity and control conditions. The pregenual cingulate BOLD response decreased from baseline during all three conditions, with the largest decrease during emotion matching (Figure 4-4A). The emotion condition did not selectively activate the amygdala.

Activations at the left and right amygdalae were found when contrasting either face matching condition (emotion or identity) with the control condition. The activation was more statistically significant at the right amygdala than the left (peak $t(11) = 7.25$ vs. 5.23 for emotion, 8.63 vs. 5.11 for identity), but the mean peak BOLD response was larger at the left amygdala than the right (0.5% vs. 0.2% for emotion, 0.7% vs. 0.3% for identity). The BOLD response appeared larger in magnitude and duration to identity than to emotion in both hemispheres. Figure 4-3 and Table 4-3 describe amygdala activation obtained using the conjunction of the contrasts (emotion - control) and (identity - control). The amygdalar clusters for the individual contrasts (emotion - control) and (identity - control) were overlapping, and are therefore not depicted separately.

Activation for both face matching conditions was also seen in the right fusiform gyrus, occipitotemporal cortex, and anterior and posterior cingulate cortices (Figure 4-4B shows cingulate activations). While the fusiform and occipitotemporal activations reflected positive BOLD responses that were larger during face matching than during the control condition, the

anterior and posterior cingulate BOLD responses were negative during the control condition and remained near baseline levels during the emotion and identity conditions.

The control condition activated extensive, bilateral regions of the occipital and parietal cortex and smaller clusters within the right anterior insula, left collateral sulcus, left fusiform gyrus, and left superior frontal sulcus. These activations reflected positive BOLD responses that were larger during pattern matching than during identity and emotion matching.

Habituation was also investigated by looking for changes over time in the behavioral and BOLD responses. Neither accuracy nor response time showed significant changes over time, indicating that fatigue did not occur. The response to emotion at the left amygdala decreased markedly over repeated blocks and showed a significant, negative correlation with time (Table 4-4, Figure 4-5). Other regions showing significant modulation of peak amplitude of the BOLD response with time are described in Table 4-4, with the slope of the linear regression representing BOLD modulation. Note that in some regions the response increased over time, and that compared with the change in response at the amygdala, the next largest modulation was only about half the size.

Discussion

Contrary to the "emotion processor" hypothesis, this study found equal amygdala activation for emotional and neutral face matching. Hariri et al. (2000) proposed that the amygdala encoded emotion at an associative level during matching of emotional faces, but the current findings require further explanation. Sander et al. (2003) describe the amygdala as a system for relevance detection stating, “An event is relevant for an organism if it can significantly influence (positively or negatively) the attainment of his or her goals.” This definition may be a more useful starting point for interpretation of the present study.

Relevance Detection Activates the Amygdala

While Hariri and colleagues acknowledge that the blocked design of their task limits its ability to investigate processing of specific emotions (Hariri et al., 2002c), they have demonstrated that matching facial expressions robustly activates the amygdala, and have successfully employed the task to probe the effects of genetics (Hariri et al., 2002b;Pezawas et al., 2005), drugs (Hariri et al., 2002a;Tessitore et al., 2002), and aging (Tessitore et al., 2005) on the amygdala and an associated affective network.

Previous studies have shown amygdala activation to neutral faces based on gaze direction, novelty, and race (Sander et al., 2003). To our knowledge the only face matching study with neutral faces investigated the effect of matching neutral faces by race (Lieberman et al., 2005). In the current study, however, matching neutral faces activated the amygdalae as much as matching emotional faces without the addition of relevance from gaze, race, etc. It seems that the matching task itself adds relevance to neutral face stimuli. Viewed alone, neutral faces would be expected to have less inherent relevance than emotional faces, but during a matching task, they must acquire task-related, goal-oriented relevance. That is, one face becomes the "right" face, while the other face becomes the "wrong" face. If we accept that the matching task itself evokes amygdalar relevance detection, we must still account for the absence of additional activation of the amygdala by emotional content, and for the apparent lack of relevance detection during the control condition.

Emotion Processing at the Amygdala Habituates

At the left amygdala, the mean amplitude of the BOLD response decreased over time for emotion but not identity matching. This suggests that while both face matching conditions activate the amygdala, there is still a distinct pattern of emotion processing at the left amygdala. Figure 4-5 shows that the initial BOLD response at the left amygdala is larger to emotion than to

identity, but then rapidly diminishes. Although the emotion condition initially evokes greater left amygdalar activation than the identity condition, habituation apparently prevented this difference from being detected with the statistical approach used in this study.

A previous study found greater habituation to repeated, passively viewed faces in the right amygdala compared with the left, and that activation in the left amygdala distinguished fearful and happy faces (Wright et al., 2001). The authors described the right amygdala as a rapid but general relevance detector, and the left amygdala as slower but capable of distinguishing emotional valence. The current findings fit this model but do not test it explicitly. It is possible that habituation of the right amygdalar response in the current study occurs rapidly within the first block, making it difficult to detect. An event-related study of habituation during explicit and incidental emotion processing may shed more light on the lateralization of the speed and specificity of emotion processing at the amygdala.

An earlier study divided the left amygdalar response to unconsciously perceived emotional faces into a ventral valence processing domain and a dorsal salience processing domain (Whalen et al., 1998b). Since the face matching task does not investigate differences in valence, this may explain the dorsal location of the amygdalar activation in the current study.

Spatial Processing Bypasses the Amygdala During the Control Condition

Although the control condition involved pattern matching it elicited no response from the amygdala compared with rest. While face matching activated the ventral visual pathway (in particular the right fusiform gyrus), pattern matching activated mostly the dorsal visual pathway (the interior parietal lobule and intraparietal sulcus bilaterally, see Table 4-3). Several participants described a pattern-matching strategy of aligning the white squares in the probe and target patterns. It is possible that this spatial alignment operation may be performed by the dorsal visual pathway, avoiding the need for relevance detection by the amygdala.

However, it is not clear whether the amygdala detects relevance in visual information using the categorical, ventral pathway exclusively. Adolphs and colleagues reported initial evidence for emotion processing via the dorsal visual pathway in one patient with extensive encephalitis lesions of the ventral surface. This patient was impaired at recognizing emotions in facial expressions, but could recognize emotions when they were acted out (Adolphs et al., 2003). We are unaware of any functional imaging study showing amygdala activation via the dorsal visual pathway.

Cognitive Processing During Emotion Matching

The emotion condition selectively activated a region of the left inferior frontal sulcus (Figure 4-2). A previous study found activation of the corresponding region in the right hemisphere to explicit, but not incidental, evaluation of facial expressions (Gorno-Tempini et al., 2001). Activity in this region of the left hemisphere has been associated with word reading (Matsuo et al., 2003) and with action recognition (Hamzei et al., 2003). Since several subjects in the current study reported mentally naming the target emotion, the observed left-sided activation may reflect covertly naming or "reading" facial expressions, especially since unfamiliar neutral faces and pixilated patterns are not easily named. While hemispheric differences in amygdala activity have been hypothesized to result from ispsilateral prefrontal efferents (Irwin et al., 2004), and Hariri and colleagues demonstrated inhibition of the amygdala by the prefrontal cortex during emotion labeling (Hariri et al., 2000), it seems unlikely that the prefrontal activation to emotion matching in the current study is responsible for the habituation of the left amygdala. The active region in the current study is in the left dorsolateral prefrontal cortex, and corresponds neither with the ventromedial prefrontal region associated with fear extinction in rats (Quirk, and Gehlert, 2003), nor with the right ventrolateral region described by Hariri et al. (2000). The correlation between the signal time course at the left amygdala and left inferior

frontal sulcus was small and not significant (r = 0.16), implying that these regions are not functionally connected during this task.

While identity matching requires a simple, perceptual match, emotion matching requires additional categorical processing (reflected in increased reaction time and decreased accuracy [Table 4-1]). This cognitive component may indirectly link prefrontal activation and amygdalar habituation during the emotion condition. The left amygdala's BOLD response to the emotion condition decreases to below the level of its response to the identity condition (Figure 4-5 A+B). Thus increased cognitive processing during the emotion condition may inhibit the amygdala, resulting in lower "baseline" (task-related) activation once the effect of emotion has habituated. We investigated the effect of task difficulty with a multivariate ANOVA using task condition (emotion, identity, or control) and block order (1st, 2nd, 3rd, or 4th exposure to block) to predict mean peak BOLD response in the prefrontal cortex and amygdala. Introducing reaction time as a covariate measure of task difficulty did not modulate the effect of task condition on mean peak BOLD response. We conclude that the observed brain activity was not quantitatively linked with task difficulty in the present study.

Negative BOLD Responses

Of additional interest are the negative BOLD responses found at the anterior and posterior cingulate cortices (Figure 4-4). These regions have been described as part of a network that is active during rest and deactivated by a variety of tasks (McKiernan et al., 2003). Because this network deactivated to both visual and auditory tasks (proportionate to difficulty in the latter case), McKiernan et al. (2003) speculated that it mediates attention-dependant processing during the conscious resting state, including monitoring emotional state. The posterior cingulate and subgenual anterior cingulate deactivated during the control condition alone, whereas the pregenual anterior cingulate deactivated during all three conditions, with the greatest decrease

for emotion. It is possible, therefore, that the posterior cingulate and subgenual anterior cingulate are involved in similar functions at rest and during the emotion and identity conditions. Conversely, matching emotions decreases activity in the pregenual cingulate cortex. A recent meta-analysis found that the peri-genual anterior cingulate cortex was activated in emotional studies and deactivated in cognitive studies (Bush et al., 2000). Furthermore, the "emotional" cingulate interacts strongly with the amygdala (Pezawas et al., 2005). Because the emotion condition involves a cognitive operation on emotional stimuli, the responses we observed at the pregenual cingulate cortex have several possible interpretations. The larger deactivation may reflect a coincidental need for increased attentional resources elsewhere or alternatively, deactivation of the pregenual cingulate may be necessary for emotional processing in the matching task.

Complex Contributions to Amygdala Activation

Activation of the amygdala by emotional face matching appears to reflect a combination of processes. Both the identity and the emotion task involve relevance detection at the amygdala simply because a choice between two faces must be made, while the control condition appears to utilize a separate spatial processing network. It appears that additional left amygdalar activation due to emotional content was not detected because of rapid habituation. Selective activation of the left inferior prefrontal sulcus and habituation of the left amygdala during the emotion condition may be indirectly linked via the common influence of increased top-down processing during emotion matching. We conclude that activation of the amygdala to the emotional face-matching task cannot be interpreted as bottom-up emotion processing alone, but likely involves more general relevance detection involved in perceptual matching.

Table 4-1 Behavioral data

Task condition	Response time (ms)	Accuracy (%)
Control	1625	86.1
Identity	* 1155	* 99.7
Emotion	1704	90.6

* Significantly different to both control and emotion, $p < 0.001$, unpaired Student's t-test.

Table 4-2 Clusters of activation for [(Emotion - Identity) ∩ (Identity - Control)]

Region	Side	BA	x	y	z	Size	t(11)
Inferior frontal sulcus	L	9, 44	-45	15	30	183	4.8
Precentral gyrus	R	4	49	-4	52	144	6.0
Pregenual cingulate cortex	L	24, 32	-4	37	10	169	-5.2
Transverse temporal gyrus	L	41	-52	-29	15	210	-6.2

Only clusters >100 voxels shown except for *a priori* region (amygdala). L: left, R: right. BA: Brodmann's Area. X, Y and Z refer to Talairach co-ordinates. Size: number of $1mm^3$ voxels. t: random effects statistical score (degrees of freedom). Negative t values indicate deactivation. ∩ denotes conjunction of two contrasts.

Table 4-3 Clusters of activation for [(Emotion - Control) ∩ (Identity - Control)]

Region	Side	BA	x	y	z	Size	t(11)
Amygdala	R		22	-7	-10	487	6.0
Amygdala	L		-9	-3	-10	35	6.0
Subgenual cingulate cortex	R	32	4	33	-10	103	6.6
Fusiform gyrus	R	37	39	-42	-19	198	5.1
Inferior temporal sulcus	R	37	49	-70	-3	200	7.6
Middle temporal gyrus	R	39	53	-59	9	883	8.6
Posterior cingulate gyrus	R	23	1	-55	23	1491	6.0
Insula	R		32	20	3	122	-6.5
Collateral sulcus	L	35, 36	-26	-40	-9	114	-6.8
Fusiform gyrus	L	19, 37	-24	-58	-12	126	-6.7
Inferior parietal lobule	R	40	40	-37	36	350	-6.6
Inferior parietal lobule	L	40	-33	-46	40	404	-6.2
Intraparietal sulcus	R	7, 19	22	-68	42	3344	-5.9
Intraparietal sulcus	L	7, 19	-17	-68	42	1081	-6.6
Middle occipital gyrus	R	19	34	-79	13	870	-5.3
Middle occipital gyrus	L	19	-25	-74	22	1015	-5.6
Superior frontal sulcus	L	6	-19	-3	55	176	-6.1

Only clusters >100 voxels shown except for *a priori* region (amygdala). L: left, R: right. BA: Brodmann's Area. X, Y and Z refer to Talairach co-ordinates. Size: number of $1mm^3$ voxels. t: random effects statistical score (degrees of freedom). Negative t values indicate deactivation. ∩ denotes conjunction of two contrasts.

Table 4-4 Regions showing significant modulation of BOLD response

Region	BOLD modulation (% / run)		
	Emotion	Identity	Control
L amygdala	* -1.39	-0.14	-0.53
L transverse temporal gyrus	* -0.52	0.33	-0.58
R amygdala	0.14	* 0.40	-0.22
R middle temporal gyrus	0.02	* 0.31	-0.12
B posterior cingulate cortex	-0.32	* 0.76	-0.45
L pregenual cingulate cortex	-0.43	0.31	* -0.50

Values represent difference in peak BOLD activation (% signal change) over one run (four blocks of each condition). * significant correlation, $p < 0.05$.

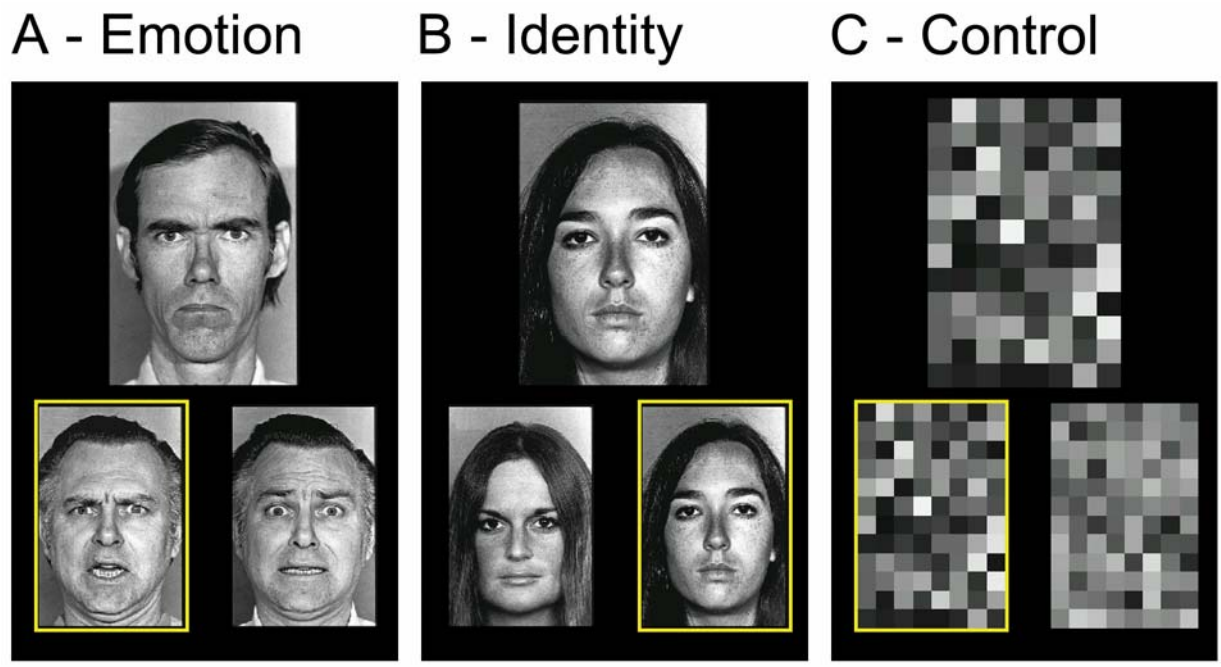

Figure 4-1 Matching task paradigm. Participants had to select which of the lower two probe images matched the upper target image. The selected probe was outlined in yellow. Faces were matched by emotion (A) or identity (B) and in the control condition (C), participants matched pixilated patterns.

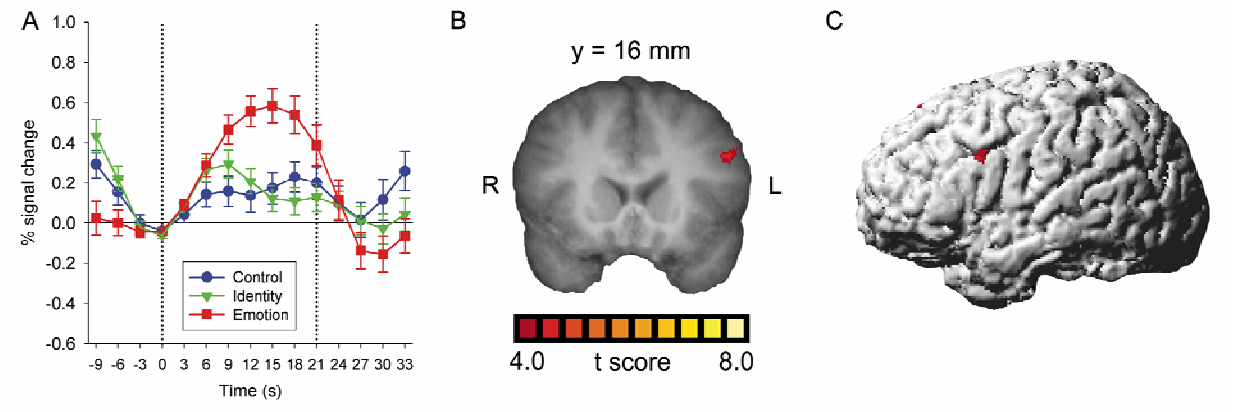

Figure 4-2 Selective response to emotion at the left inferior prefrontal sulcus. A: BOLD response; vertical dotted lines indicate beginning and end of block; error bars denote standard error of mean. B: Cluster of activation for [(Emotion - Identity) ∩ (Identity - Control)] with a threshold of $t(11) > 4.0$; slice location given in Talairach co-ordinates; slice in radiological convention (Table 4-2). C: Left inferior prefrontal sulcus activation for the group illustrated on a rendered 3D brain from a single participant.

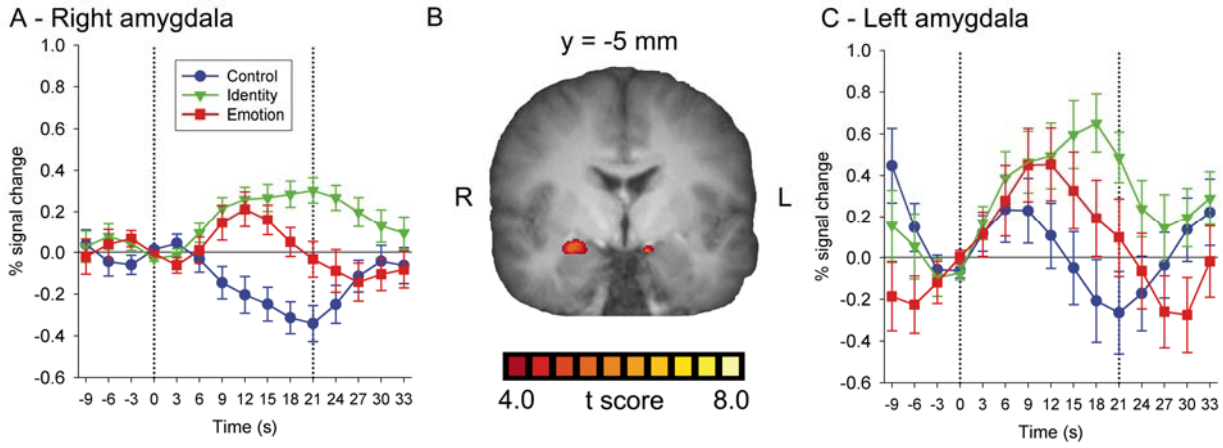

Figure 4-3 Response to face matching at the left and right amygdala. A+C: BOLD responses; vertical dotted lines indicate beginning and end of block; error bars denote standard error of mean. B: Clusters of activation for [(Emotion - Control) ∩ (Identity - Control)] with a threshold of $t(11) > 4.0$; slice location given in Talairach co-ordinates; slice in radiological convention (Table 4-3).

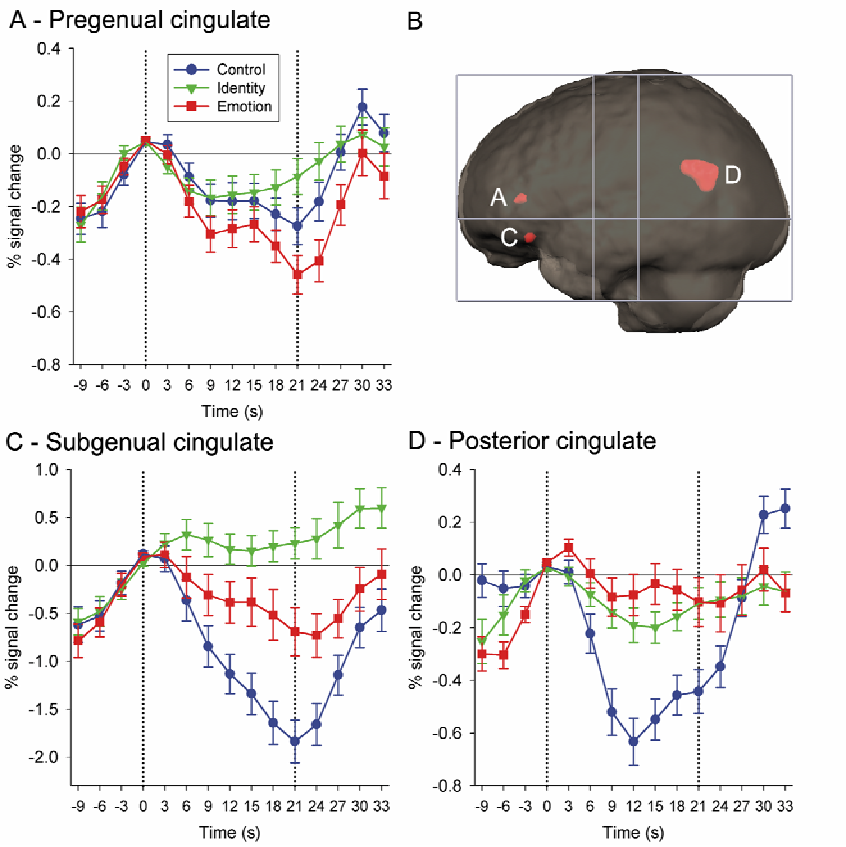

Figure 4-4 Regions of deactivation. B: "Glass brain" showing clusters of activation with a threshold of t(11) > 4.0 in the cingulate cortex (Tables 4-2 & 4-3). Gray borders denote the anterior and posterior commisures and the borders of the cerebrum. A, C+D: BOLD responses; vertical dotted lines indicate beginning and end of block; error bars denote standard error of mean. A: Pregenual cingulate cortex (-4, 37, 10). The BOLD response is negative for all three conditions, with a significantly greater decrease for emotion. C+D: Subgenual cingulate cortex (4, 33, -10) and posterior cingulate cortex (1, –55, 23). The BOLD response is negative in the control condition, but remains at baseline for emotion and identity.

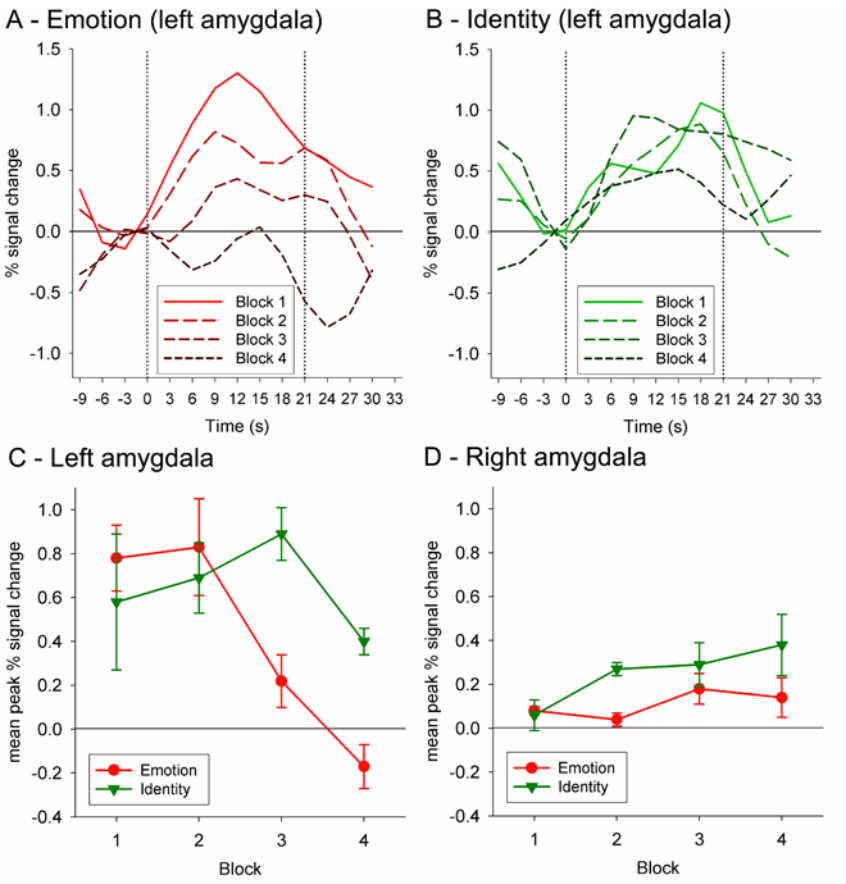

Figure 4-5 Habituation. A+B: The BOLD response at the amygdala decreases over time in the emotion condition but not in the identity condition. BOLD responses are derived from left amygdala activation clusters for the contrasts (emotion - control) (A) and (identity - control) (B). C+D: The peak BOLD response habituates only at the left amygdala in the emotion condition. Peak response = mean % signal change values for 9 sec – 18 sec time points. Responses derived from left and right amygdala clusters for the contrast [(Emotion - Control) ∩ (Identity - Control)] (Table 4-3).

CHAPTER 5
DISSOCIATING EVENT-RELATED RESPONSES TO TOP-DOWN AND BOTTOM-UP EMOTION PROCESSING

Conscious rationalization of emotional stimuli may modulate automatic physiological responses to stimulus content. Functional neuroimaging studies have investigated top-down modulation of emotion using tasks that require participants to rate their responses to emotional pictures. These studies suggest that the amygdala and insula may mediate bottom-up responses to the content of emotional scenes, and that these responses may be modulated by top-down processing mediated by the anterior cingulate cortex (ACC) and medial prefrontal cortex. We attempted to replicate these findings using an optimized paradigm design. Pleasant and unpleasant pictures were rated using emotional and non-emotional rating scales: either the pleasantness of the picture, or the frequency of its appearance on television. Stimulus content and tasks instructions were randomized on a trial-by-trial basis, preventing expectancy of emotional content, and equalizing the timing of bottom-up and top-down responses. Factorial analysis was used to separate the main effects of bottom-up and top-down processing and their interaction. The amygdala responded to unpleasant pictures during both tasks. The orbitofrontal cortex and amygdala responded during emotional rating of both pleasant and unpleasant pictures. The ACC responded to both stimulus content and task demands. No significant interaction effects were found. These results show that an event-related picture rating task may dissociate bottom-up processing in the amygdala from top-down processing in the orbitofrontal cortex and insula.

Introduction

Psychological and neuroanatomic evidence suggests that responses to emotional stimuli may be mediated both by automatic, bottom-up processes and by intentional, top-down processes (Eysenck, and Keane, 2000). Zajonc proposed the affective primacy hypothesis, which states the appraisal of emotional stimuli is rapid and automatic (Zajonce, 1980, quoted in Eysenck &

Keane, 2000). He supported this hypothesis by showing that subliminally presented emotional faces may influence pleasantness ratings of a subsequently presented Chinese pictogram. Lazarus, on the other hand, argued that emotional responses are influenced by conscious, contextual appraisal (Lazarus, 1982, quoted in Eysenck & Keane, 2000). He supported this hypothesis by demonstrating that physiological responses to a disturbing film varied depending on the narrative that accompanied the film. Although these hypotheses appear to disagree, later theorists proposed that emotional experience may result from the appraisal of a stimulus by both bottom-up and top-down processes (Ellsworth, 1994). Anatomical evidence supports multiple, interacting pathways for emotional appraisal. The idea of two parallel emotion systems is supported by anatomical evidence. In rats, conditioned fear responses are dependent upon the amygdala. LeDoux showed that fear-inducing visual signals may reach the amygdala by two pathways: a fast subcortical pathway, and a slow cortical pathway (LeDoux, 2000). Subsequent functional neuroimaging studies in humans have sought to describe the roles of the amygdala and associated cortical regions in emotional appraisal.

Several investigators have investigated bottom-up and top-down components of emotional appraisal by employing emotion rating tasks. Bottom-up processes may be mediated by brain regions that respond to differences in the emotional content of stimuli. Top-down processes may be mediated by brain regions that respond only during explicit rating of emotion. By altering task demands, these studies investigated the modulation of bottom-up responses by top-down appraisal. In the earliest rating study, participants viewed blocks of mixed unpleasant and neutral pictures during positron emission tomography (PET), and rated either whether each picture was pleasant or unpleasant or whether it was indoors or outdoors (Lane et al., 1997a). Comparing emotion rating with location rating, activation was seen in a cluster spanning the anterior

cingulate cortex (ACC) and medial prefrontal cortex (PFC) (both Brodmann Area 32) and in the insula. The authors suggested that the ACC mediated internal attention specifically associated with emotion processing, and that increased activity at the insula represented amplification of interoceptive processing. This study could not investigate bottom-up responses because unpleasant and neutral pictures were intermingled. In two subsequent studies, pleasant and neutral pictures were presented separately to identify regions involved in bottom-up responses (Liberzon et al., 2000;Taylor et al., 2003). Liberzon et al. (2000) compared emotion rating with picture recognition, a cognitive task intended to draw attention away from emotion. The right amygdala was activated by unpleasant compared with neutral pictures, and activation was greater during emotion rating than during picture recognition. Taylor et al. (2003) compared emotion rating with passive viewing, in order to test whether top-down processing diminished bottom-up responses. The right amygdala and insula responded to unpleasant pictures, but the response was smaller during emotion rating than during passive viewing. The opposite effect was seen in the ACC and medial prefrontal cortex: activation by unpleasant pictures was increased during emotion rating compared with passive viewing. A later study compared emotion rating with ratings of personal relevance (Phan et al., 2004). The left amygdala responded more strongly to emotional pictures during emotion rating; however the ACC and medial PFC were deactivated during emotion rating. This finding suggests a specific role for these regions in the appraisal of self-relevance, which Zajonc and Smith (1993) defined as a component of emotional appraisal (quoted in Eysenck and Keane, 2000). This view is supported by the recent finding that the medial PFC responds more strongly when rating one's own emotions than when rating other's emotions (Ochsner et al., 2004). In summary, these studies suggest that the amygdala and insula

may mediate bottom-up responses to emotional stimulus content, and that these responses may be modulated by top-down processing mediated by the ACC.

The studies above employ several task designs. In all but one study, emotional pictures are presented in blocks, so neural responses to emotional content may be confounded with expectancy of emotion, a top-down effect. Similarly, most studies tested for interactions between bottom-up and top-down factors using single statistical contrasts, which do not account for the main effects of each factor (Friston et al., 1996). Phan et al. (2004) presented emotional pictures in a random, event-related design and tested interactions using a factorial analysis. However, their rating tasks were presented in blocks. Because the bottom-up and top-down factors varied at different time intervals, the neural responses to each factor may have unequal power. Finally, these studies used a variety of control tasks. Passive viewing fails to control for the attentional and motor components of emotion rating. Recognition and self-relevance rating include internal appraisal and thus may be confounded with emotion rating. The indoor / outdoor judgment task avoids these problems, but elicits only two categorical responses, while emotion rating tasks typically elicit responses on a continuous scale.

In the current study, we investigate bottom-up and top-down emotional appraisal using an optimized emotion rating paradigm. We employ an event-related picture rating task in which both emotional content and task instructions are randomized. By randomizing both bottom-up and top-down factors at the same temporal frequency we avoided contaminating our findings with differential signal-to-noise characteristics of event- and block-level hemodynamic responses. As a control task, participants were asked how frequently images similar to the one displayed are shown on television. We chose frequency rating as a control condition because it controls for attentional and motor components of the rating task, requires attention to external

stimulus features, and elicits a response on a continuous scale. We used factorial analysis to identify the neural responses to the task. Regions showing a main effect of stimulus content were defined as mediated bottom-up responses. Regions showing a main effect of rating task were defined as mediating top-down appraisal. Regions showing an interaction effect were defined as mediating bottom-up responses that were modulated by top-down appraisal. We tested the hypotheses that the amygdala mediates a bottom-up response that is modulated by top-down appraisal, and that the ACC mediates top-down appraisal.

Methods

Subjects

Sixteen healthy male participants gave informed consent as approved by the University of Florida's Institutional Review Board. The participants had no history of psychiatric or neurological illness, and were taking no psychotropic medication at the time of the study. One participant was excluded due to discrete head movements greater than 1mm during scanning.

Picture Rating Task Paradigm

Participants were presented with pictures from the International Affective Picture System (IAPS) (Center for the Study of Emotion and Attention [CSEA-NIMH], 2001). Below each picture, a cue instructed participants to make either an emotion or frequency rating. The emotion rating cue read, "How pleasant do you find the content of this image?" The frequency rating cue read, "How frequent do images with similar content appear on television?" The participants selected one of four responses: for emotion ratings, very unpleasant, moderately unpleasant, moderately pleasant, or very pleasant, and for frequency ratings, weekly, daily, hourly, or continuously. We selected emotionally arousing IAPS pictures and assigned them, based on the valence ratings, to two groups: pleasant or unpleasant (Lang et al., 2001). The mean ratings were, for the pleasant set, pleasure = 6.7 +/- 0.9, arousal = 4.7 +/- 1.0, and for the unpleasant set,

pleasure = 3.7 +/- 1.1, arousal = 4.8 +/- 1.3 (mean +/- standard deviation). IAPS picture codes are listed in appendix A. The trials were categorized by rating task and valence, giving four trial types: emotion rating pleasant (EP), emotion rating unpleasant (EU), frequency rating pleasant (FP), and frequency rating unpleasant (FU).

Prior to performing the task, each participant was familiarized with the task and the scanner environment by completing a training run consisting only of emotion ratings. Different sets of pictures were used for the training and task runs. The results of the training run are reported elsewhere, in a study of the effects of training upon emotional and non-emotional ratings (Li et al., 2006).

During the task run, all four trial types were presented in a random order using an event-related design. Fifteen trials of each type were presented for 3 sec each, along with 30 null trials, during which a fixation cross was displayed for 3 sec. The test run lasted 4 min 30 sec. Null trials were included in the random sequence in order to jitter the stimulus onset asynchrony (SOA) between trials. This increases the variance in the resulting fMRI response, making the response to rapid stimuli (SOA < 15 sec) detectable (Burock et al., 1998). Jittering the SOA with randomly interspersed null trials creates a geometric distribution of SOAs, which is more efficient than uniform randomization (Serences, 2004). The resulting mean SOA was 4.5 sec, with a minimum of 3 sec. This timing was chosen to minimize response attenuation when repeating emotional stimuli, but to maximize the number of trials in the run (Soon et al., 2003).

The stimuli were presented using an Integrated Functional Imaging System (IFIS, MRI Devices, Inc., Waukesha, WI) with a 7” LCD screen at 640 X 480 pixel resolution, mounted over the subject’s head and viewed using a fixed mirror. The screen subtended approximately 14° x 11° of the visual field. A PC running E-Prime (Psychology Software Tools, Pittsburgh, PA)

began presenting each task in synchronization with the first RF pulse of each scan. Responses were collected with a MRI-compatible button glove attached to the participant's right hand.

Functional Imaging Data Acquisition

Participants were scanned using a 3 Tesla Siemens Allegra scanner with a standard head coil (Siemens, Munich, Germany). Anatomic images were acquired using an MPRAGE sequence with TR = 1500 ms, TE = 4.38 ms, and flip angle = 8°. In the axial plane, 160 slices were acquired (thickness 1.0 – 1.2 mm, according to the height of the brain) with in-plane field of view 240 mm X 180 mm and matrix size 256 X 192. Functional images covering the whole brain were acquired using echo-planar imaging sensitive to blood-oxygenation level dependent (BOLD) effects, with TR = 3000 ms, TE = 30 ms, flip angle = 90°. In the axial plane, 38 slices with a thickness of 3.8 mm were aligned with the plane of the intercommissural line and had an in-plane field of view 240 X 240 mm and matrix size 64 X 64. The first two volumes of each functional run were discarded to allow for T1 equilibration. These settings have previously been shown to provide reasonable coverage of the amygdala while allowing coverage of the whole brain, and without sacrificing BOLD sensitivity (Wright, and Liu, 2005). Visual inspection of functional images showed coverage in the amygdala was adequate in ten out of fifteen participants. Because our *a priori* hypotheses predicted responses in the ACC, coverage of this region was inspected. Signal in the subgenual ACC was lost to susceptibility artifact, and responses within the affected region were discarded.

Functional Imaging Data Analysis

Data were analyzed using BrainVoyager QX version 1.7.6 (Brain Innovations, Maastricht, Holland). The functional images were coregistered with anatomic images, and normalized to Talairach space for each participant. Functional data underwent 3D motion correction, linear trend removal and slice scan time correction. The test runs underwent Gaussian spatial

smoothing using a kernel of 5.7 mm (1.5 voxels) full-width half-maximum (FWHM). The training run underwent spatial smoothing as above and temporal Gaussian smoothing using a kernel of 4 data points (12 sec) FWHM.

Task-related activity was mapped using a voxel-wise general linear modeling analysis. For both event-related and block analyses, the BOLD response to each task condition was estimated using a standard hemodynamic model (Friston et al., 1998). The estimated responses were fit to the MR signal for each individual to generate a beta weight, reflecting the magnitude of the contribution of each task type to the overall model. Using the conservative random-effects approach, statistical maps were generated by applying second-order statistics to the group's beta weights at each voxel. For the test runs, a two-way ANOVA was used to estimate separately the main effects of judgment type and emotional valence, and their interaction. This approach avoids the assumption of pure insertion, allowing the localization of neural responses that correlate with cognitive task components in a nonlinear fashion (Friston et al., 1996). In the training run a t-test was used to compare early vs. late training responses (the first two blocks of ten vs. the last two). For whole-brain analysis, thresholds were set to exclude clusters smaller than 100 mm^3 (after functional data were resampled to 1 mm resolution), and statistical scores below $F(1,14) = 12$, $p < 0.005$. For regions for which we had an *a priori* hypothesis (amygdala, OFC, and ACC) the statistical threshold was lowered to $F(1,14) = 5$, $p < 0.05$. At each region, t-score were calculated post-hoc using single statistical contrasts to indicate the direction of the main effect or interaction.

Mean BOLD responses were plotted using BrainVoyager's event-related averaging function. In the test runs, BOLD responses were calculated for each of the four trial types. In the training run, BOLD responses were plotted for early, middle, and late blocks. For each test run

event, percentage signal change was calculated relative to the signal during the two seconds prior to stimulus onset. These values were averaged by event type in a time window from –2 to 13 seconds relative to stimulus onset. Contamination from subsequent stimuli occurring within the 13-second window was eliminated in the overall average due to the jittered SOA (Dale, and Buckner, 1997). Events were not time-locked to the fMRI sampling period (3 seconds) to allow finer sampling of the BOLD response (Serences, 2004). Data therefore were resampled to 1 second resolution by interpolation. BOLD responses to the training paradigm were calculated in a similar way, but using the original sampling period. Signal change was calculated relative to the two periods preceding the start of each block (6 seconds). A time window from –10 to 36 sec was used to cover the BOLD response to the entire block.

Results

Behavioral Data

Participants rated pleasant and unpleasant stimuli appropriately. Pleasure ratings, adjusted to the standard scale used in the IAPS of 1 – 9 were significantly higher for pleasant pictures compared with unpleasant pictures (6.6 +/- 0.9 vs. 3.6 +/- 0.8, $p < 0.001$). Response times were slower during frequency rating, regardless of emotion, implying that the control task was more difficult (Table 5-1).

fMRI Data

Responses were observed in the regions for which an *a priori* hypothesis was proposed. A main effect of task was observed in the left OFC and right insula (Figure 5-1). These regions showed positive BOLD responses to emotion rating and negative responses to frequency rating. Supporting the statistical main effect, the BOLD responses appeared equal for pleasant and unpleasant pictures (Figure 5-1 B & D). The left amygdala responded selectively to unpleasant pictures (Figure 5-2). Activation was detected at reduced threshold ($F(1,14) = 5$, $p < 0.05$) due to

the *a priori* selection of this region. A positive BOLD response was seen for negative pictures during both tasks, supporting the main effect of valence. Examination of the ACC, also at reduced threshold, revealed three activations, two exhibiting a main effect of valence, and one a main effect of task (Figure 5-3). These clusters were distinct, but overlapped slightly. The BOLD responses in these regions suggested that in both clusters exhibiting a main effects of valence, the result was driven by positive responses to pleasant pictures. In the cluster exhibiting a main effect of task, the result appeared to be driven by a positive response to emotion rating (Figure 5-3 B-D). However, the BOLD response curves were not as clearly separated in these regions as they were in the OFC, insula, and amygdala.

Regions for which we proposed no *a priori* hypothesis also showed interaction effects (Table 5-2) and main effects of valence (Table 5-3) and task (Table 5-4). An interaction between task and valence was located between the left insula and temporal operculum, a region anterior and posterior to the main effect of task at the right insula. The BOLD responses at this region (not shown) were more variable than at other regions, and did not have a typical curve shape, suggesting they may have arisen from the nearby middle cerebral artery. Several regions showed a main effect of valence, namely the bilateral posterior cingulate cortex, postcentral gyrus, and posterior fusiform cortex. A main effect of task was seen in the bilateral parieto-occipital sulcus, showing greater BOLD responses to the frequency task, regardless of valence. Several other regions showed greater responses to frequency ratings, predominantly in the left PFC, including premotor cortex and supplementary motor area (SMA).

Discussion

The aim of the current study was to identify regions of the brain involved in bottom-up and top-down emotional appraisal. We pursued this aim using an event-related, factorial design to identify regional brain responses that varied with stimulus valence, task instructions, or an

interaction of both factors. Previous studies implicated the amygdala, insula, anterior cingulate cortex (ACC), and orbitofrontal cortex (OFC) in task involving emotional ratings. The current study found responses to the emotion rating task at the OFC and insula, and to stimulus content at the amygdala. Both effects, and an interaction between stimulus content and task instructions, were seen in different subdivisions of the ACC.

Top-down Appraisal in the OFC and Insula

Rating emotion activated the left OFC and bilateral insulae. These regions showed a main effect of task, and as the BOLD responses confirm, responded equally to both pleasant and unpleasant stimuli. These findings suggest that both regions are implicated in top-down emotional appraisal, independently of bottom-up responses.

Previous studies associated the OFC with emotional rating of pictures, odors and words (Cunningham et al., 2004;Liberzon et al., 2000;Royet et al., 2003). In the picture rating study, the OFC showed an increased response to unpleasant relative to pleasant pictures during emotion rating, but not during picture recognition (Liberzon et al., 2000). However, direct comparison of the rating and recognition tasks revealed no interaction effect. Furthermore the region of the OFC that was activated (1, 23, -18) was medial to the cluster found in the current study (-26, 35, -7), therefore a direct comparison of the two responses may not be meaningful. Studies in monkeys have shown that OFC neurons respond to the reward value of abstract objects, even when the objects' reward associations are changed (Rolls, 1999). In humans, lesions of the OFC impair social functioning, decision making, and long-term planning (Damasio, and Van Hoesen, 1983). The OFC is commonly activated in reward studies, in which participants learn to associate abstract stimuli with monetary value. It is possible that in the current study, the response in the OFC reflects an intentional, explicit judgment of stimulus value (O'Doherty, 2004).

The insula receives sensory input from the viscera, and has been associated with awareness of internal sensations and with the emotion of disgust (Critchley, 2005;Phillips et al., 1997;Wright et al., 2004). Although the insula appears closely involved in the visceral component of emotion, and therefore would be associated with bottom-up emotional appraisal, Adolphs (2002) proposed a sequence of regional brain responses during facial emotion processing in which the insula responds last, possibly corresponding with conscious awareness of emotion (Adolphs, 2002). A previous study interpreted activation of the insula during emotion rating as an amplification of interoceptive cues, but this study's design did not assess the effect of the stimulus content (Lane et al., 1997a). A later study showed an increased response at the insula to unpleasant versus neutral pictures; the response decreased during emotion rating compared with passive viewing (Taylor et al., 2003). In a third study, the insular response was proportional to emotional intensity[1], and was increased during both emotion rating and self-relatedness rating (Phan et al., 2004). In Phan et al. (2004), all insula responses were anterior to the response reported here, whereas in Taylor et al. (2003), the insula response corresponded with the current study. However, Taylor et al. compared unpleasant and neutral pictures, whereas the current study compared unpleasant and pleasant pictures. The two picture sets used in the current study thus differed in pleasure ratings, but not in arousal ratings. Therefore it is possible that our equal insula responses reflected our two picture sets' equal arousal scores. Thus the main effect of task in the current study may in fact reflect top-down modulation of bottom-up responses that are equal to both pleasant and unpleasant pictures. Taylor et al. (2003) found that the insular response was decreased during emotion rating, whereas in the current study it was increased. This may be explained by the use of different control conditions in the two studies.

[1] In Phan et al. (2004), intensity is distinct from arousal, and refers to a measure derived from the pleasure scale, where high intensity means high or low pleasure, and low intensity means moderate pleasure.

Relative to emotion rating, frequency rating may decrease emotional responses, whereas passive viewing may increase them. The above hypotheses could be tested in a future study combining three picture conditions (pleasant, neutral, and unpleasant) and three task conditions (emotion rating, passive viewing, and a non-emotional rating task).

Bottom-up Processing in the Amygdala

The left amygdala responded selectively to unpleasant pictures, regardless of task instructions (Figure 5-2). Bottom-up processing in the amygdala is consistent with previous studies, although these tended to report responses in the right amygdala that were modulated by different rating tasks (Liberzon et al., 2000;Taylor et al., 2003), or constant across tasks (Phan et al., 2004). The difference in laterality between previous studies and the current study, and the lack of modulation of the amygdala response, may be due to the training period that preceded testing. A previous study of facial expression recognition reported that amygdala responses shifted over time from right to left, which the authors suggested represented a shift in processing style (Gur et al., 2002). Other studies supported differences in processing styles in the right and left amygdala. Lesions of the right amygdala impaired general, autonomic responses to emotional faces, but lesions of the left amygdala impaired recognition of specific facial expressions (Glascher, and Adolphs, 2003). While the right amygdala responded to subliminally-presented facial expressions of fear, the left amygdala responded to seen faces (Morris et al., 1999). The right amygdala responded preferentially to emotional pictures, whereas the left amygdala responded preferentially to words (Markowitsch, 1998;Phelps et al., 2001). The left amygdala response in the current study may therefore represent comparatively more conscious and specific processing of emotion than the right amygdala response reported in previous emotion rating studies. A behavioral study of the effects of training in emotional rating showed that after training, individuals are more likely to make emotional ratings spontaneously when

presented with an image and allowed to respond freely (Li et al., 2006). The author's suggested that explicit emotion rating may become proceduralized, or implicitly learned. Thus, in the current study, training in emotion rating may have produced an automatic, early appraisal of emotional valence regardless of whether an emotion rating was required. The left amygdala response may therefore mediate implicitly learned emotion rating that is immune to modulation by task instructions. Future studies may specifically investigate how the neural correlates of emotion rating change over time, and may also investigate the differential sensitivity of the left and right amygdalae to modulation by different rating tasks.

Mixed Responses at the Anterior Cingulate Cortex

Adjacent regions of the ACC responded to emotion ratings and to pleasant pictures (Figure 5-3). While previous studies associated this region with top-down processing, the current findings suggest that this region mediates multiple levels of emotion processing. Both types of responses reported here have been reported in previous studies. The ACC responded during emotion ratings (compared with a control task) in several previous studies, although the location of the responses in these studies was more dorsal than those in the current study (Cunningham et al., 2004;Lane et al., 1997a;Ochsner et al., 2004). Several other studies have reported responses to happy mood induction in the pregenual ACC, the same region that in the current study responded to pleasant pictures (see Vogt, 2005). Researchers are currently investigating the roles of different subdivisions of the ACC in emotion processing (Bush et al., 2000;Vogt, 2005). The current findings implicate different subdivisions in bottom-up and top-down emotion processing. However, because the BOLD responses in the ACC were less clearly separated than the responses in the OFC, insula, and amygdala (Figure 5-3 B-D), the statistical findings are supported with less confidence in this region. Previous studies reported a strong top-down response in the ACC (Cunningham et al., 2004;Lane et al., 1997a;Ochsner et al., 2004). The

apparently weak top-down response in the current study may be due to the event-related design. Previous studies varied rating tasks in a block-related fashion, and it is possible that clearly differentiated responses in the ACC to top-down emotion processing require the establishment of a cognitive set over several stimuli, as is thought to occur during block presentation. The current study varied both emotional content and task instructions at the event level so that both factors would elicit responses of the same temporal magnitude, and therefore have comparable statistical power. The stimulus onset asynchrony (mean 4.5 seconds) was previously shown to maximize the efficiency of detecting responses to emotional faces, a bottom-up effect (Serences, 2004). Future studies may investigate most efficient timing of stimuli for the detection of top-down effects.

Response to Frequency Rating in the Parietal Cortex

Frequency judgments activated the bilateral parieto-occipital sulcus. The precise region of activation in the current study was at the border of the parieto-occipital sulcus and the intraparietal sulcus. Bilateral parietal activations at these co-ordinates were seen in a previous study during the spatial control task (indoor / outdoor judgment) (Lane et al., 1997a). Although the parietal lobe is generally associated with the dorsal visual pathway, which processes spatial location, this region is not associated with a single, specific function. The parietal cortex is an associative region, receiving multimodal inputs, and being activated by a wide range of cognitive tasks. The region activated in the current study may correspond with the caudal intraparietal sulcus (CIP), which studies in monkeys and humans have implicated in grasping, and in processing object orientation (Culham, and Kanwisher, 2001). The parietal cortex is also implicated in arithmetic tasks. A recent metanalysis posits a mental “number line” represented at the horizontal section of the intraparietal sulcus (Dehaene et al., 2004). While this section is anterior to the region found in the current study, the above authors propose that additional

attentional orientation on the mental number line may occur more posteriorly. Thus it is possible that the activation in the current study reflects the numerical approximation involved in the frequency judgment task.

Summary and Conclusions

The aim of this study was to use an emotion rating task to dissociate the neural correlates of automatic, bottom-up responses to pictures' emotional content and of voluntary, top-down responses during explicit emotion rating. This was the first study of its kind to employ an event-related, factorial design. Event-related stimulus presentation eliminated the effect of anticipation of either task or emotion. Factorial design and analysis controlled for non-linear differences in the neural responses to each task condition by accounting for the main effect of emotional valence (pleasant or unpleasant), the main effect of task (emotion or frequency rating), and interactions between the two (representing top-down modulation of responses to stimulus content). In agreement with previous studies, we demonstrated a top-down response in the orbitofrontal cortex, and a bottom-up response in the left amygdala. Unlike previous studies, which found top-down responses in the ACC, and top-down modulation of bottom-up responses in the insula, we found both top-down and bottom-up responses in the ACC, and a top-down response in the insula. These results demonstrate the potential of fMRI to distinguish bottom-up and top-down emotion processing at the event level. The pleasant and unpleasant stimuli used in the current study varied in emotional valence but not in arousal. Future studies may test whether including neutral pictures may reveal top-down modulation of bottom-up responses in the insula that are driven by differences in arousal. The timing of stimulus presentation used in the current study was previously shown to elicit reliable bottom-up responses. Future studies may investigate whether a slower event-related design or a blocked design is necessary to elicit reliable top-down responses in the ACC. With these improvements, this emotion rating paradigm

may be used to study differential alterations in top-down and bottom-up pathways that may be seen in patients with affective disorders.

Table 5-1 Response time in milliseconds (standard deviation)

Stimulus valence	Rating task	
	Emotion	Frequency
Pleasant	1902 (295)	2088 (263)
Unpleasant	1899 (265)	2087 (295)

Responses < 500 ms were excluded as they most likely reflected carried-over late responses to previous trials. ANOVA of task and valence revealed a significant main effect of task ($F(1, 14) = 36.5$, $p < 0.001$).

Table 5-2 Clusters of activation for interaction of valence and task.

Region	Side	BA	x	y	z	Size	F(1,14)	t(14)
Subgenual cingulate cortex	L	25	*-4*	*22*	*-9*	*254*	*10.9*	*2.4*
Insula / MCA	L		36	11	-15	109	21.8	5.1

Only clusters > 100 mm^3 shown. BA: Brodmann Area. X, Y and Z refer to Talairach co-ordinates (mm). Size: number of $1mm^3$ voxels. F: result of whole-brain ANOVA (degrees of freedom); score is taken from the peak voxel of the cluster. t: random effects statistical score for post-hoc cluster-based contrast [(EU+FP) - (EP+FU)] (degrees of freedom). EU: emotion rating on unpleasant pictures, EP: emotion rating on pleasant pictures, FU: frequency rating on unpleasant pictures, FP: frequency rating on pleasant pictures. BA: Brodmann area. MCA: middle cerebral artery. *Italic text: a priori region of interest, tested at lower statistical threshold.*

Table 5-3 Clusters of activation for main effect of valence.

Region	Side	BA	x	y	z	Size	F(1,14)	t(14)
Pregenual cingulate cortex	R	32	*8*	*39*	*18*	*1092*	*15.0*	*2.9*
Postcentral gyrus	L	2	-39	-31	56	397	16.4	4.2
Posterior cingulate	R	30	8	-52	12	583	23.1	4.5
Posterior cingulate	L	30	-10	-53	12	288	20.5	4.4
Amygdala	L		*-21*	*-7*	*-10*	*203*	*13.0*	*-2.2*
Inferior occipital gyrus	L	18	-39	-75	-8	362	24.9	-5.3

Only clusters > 100 mm^3 shown. BA: Brodmann Area. X, Y and Z refer to Talairach co-ordinates (mm). Size: number of $1mm^3$ voxels. F: result of whole-brain ANOVA (degrees of freedom); score is taken from the peak voxel of the cluster. t: random effects statistical score for post-hoc cluster-based contrast [(EP + FP) - (EU + FU)] (degrees of freedom); positive values: greater response to positive pictures, negative values: greater response to negative pictures. EU: emotion rating on unpleasant pictures, EP: emotion rating on pleasant pictures, FU: frequency rating on unpleasant pictures, FP: frequency rating on pleasant pictures. BA: Brodmann area. *Italic text: a priori region of interest, tested at lower statistical threshold.*

Table 5-4 Clusters of activation for main effect of task.

Region	Side	BA	x	y	z	Size	F(1,14)	t(14)
Orbitofrontal cortex	L	11	-26	35	-7	315	19.0	4.8
Pregenual cingulate cortex	B	24	*-1*	*34*	*10*	*588*	*10.4*	*3.4*
Insula	R		42	-1	5	879	26.1	4.6
Insula	L		-40	-5	5	151	16.7	4.3
Superior temporal gyrus	L	42	-61	-8	9	104	17.9	4.6
Parahippocampal gyrus	R	28	16	-14	-21	207	22.0	5.7
Callosomarginal gyrus	L	4, 31	-7	-33	46	103	19.5	4.3
Precuneus	R	7	14	-62	46	172	25.0	4.9
Inferior frontal gyrus	L	45	-48	34	8	245	20.3	-4.7
Middle frontal gyrus	R	9	47	29	33	278	21.6	-5.1
Medial frontal gyrus	L	6, 8	-3	19	49	651	21.4	-4.6
Inferior frontal gyrus	L	9	-45	16	28	1130	37.0	-5.3
Middle frontal gyrus	L	6	-34	3	52	1060	28.4	-5.1
Superior temporal sulcus	L	21, 22	-61	-40	0	1013	30.6	-5.0
Parieto-occipital sulcus	L	7, 19	-37	-67	41	1680	22.9	-5.2
Parieto-occipital sulcus	R	7, 19	40	-70	40	1478	21.6	-5.4

Only clusters > 100 mm^3 shown. BA: Brodmann Area. X, Y and Z refer to Talairach co-ordinates (mm). Size: number of $1mm^3$ voxels. F: result of whole-brain ANOVA (degrees of freedom); score is taken from the peak voxel of the cluster. t: post-hoc cluster-based contrast [(EP + EU) - (FP + FU)] (degrees of freedom); positive scores: greater response to emotion rating task, negative scores: greater response to frequency rating task. EU: emotion rating on unpleasant pictures, EP: emotion rating on pleasant pictures, FU: frequency rating on unpleasant pictures, FP: frequency rating on pleasant pictures. BA: Brodmann area. MCA: middle cerebral artery. *Italic text: a priori region of interest, tested at lower statistical threshold.*

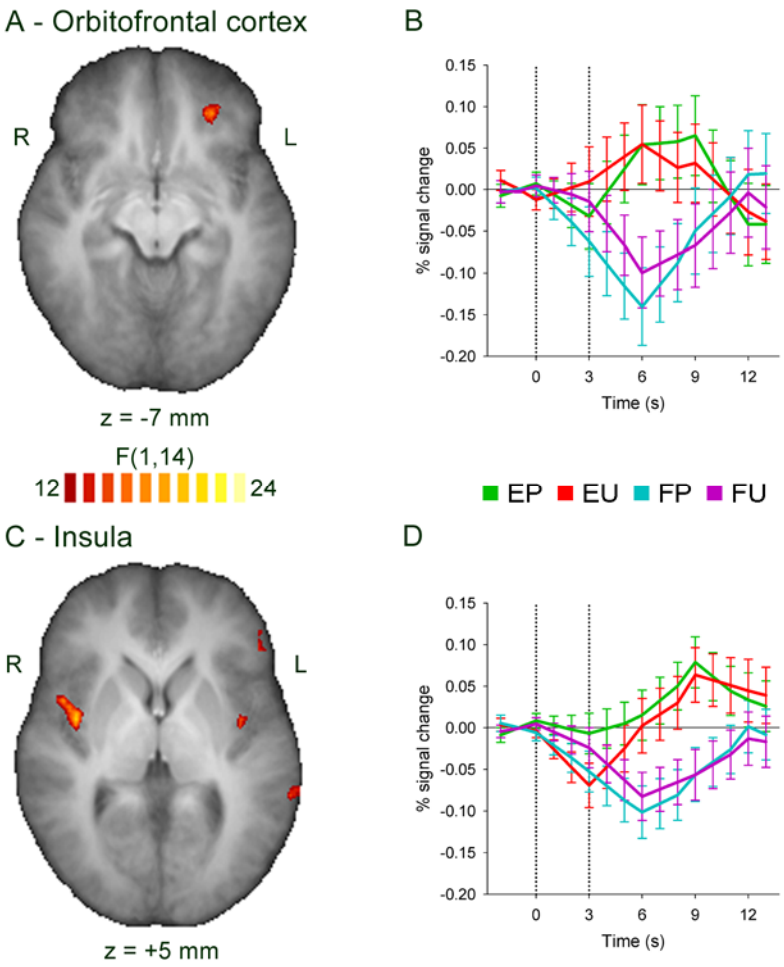

Figure 5-1 Main effect of task. A & C) statistical maps showing a main effect of task in the right insula (A) and the left OFC (C). L: left. R: right. Z: position of axial slice in Talairach space. Color scale indicates F score on two-way ANOVA (see methods). B & D) BOLD responses in the right insula (B) and left OFC (D). Error bars show standard error. Vertical lines show beginning and end of trial. EP: emotion rating on pleasant pictures, EU: emotion rating on unpleasant pictures, FP: frequency rating on pleasant pictures, FU: frequency rating on unpleasant pictures.

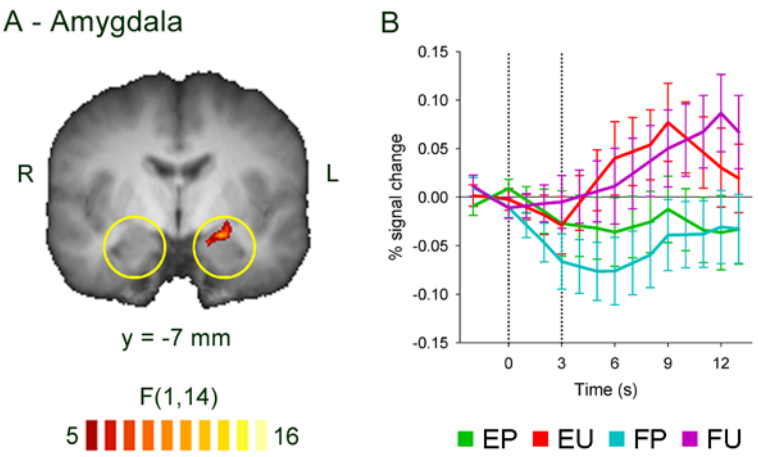

Figure 5-2 Main effect of valence. A) reduced-threshold statistical map showing a main effect of valence at the left amygdala. Responses are only shown inside the *a priori* regions of interest (yellow circles). L: left. R: right. Y: position of coronal slice in Talairach space. Color scale indicates F score on two-way ANOVA. B) BOLD response in the left amygdala. Error bars show standard error. Vertical lines show beginning and end of trial. EP: emotion rating on pleasant pictures, EU: emotion rating on unpleasant pictures, FP: frequency rating on pleasant pictures, FU: frequency rating on unpleasant pictures.

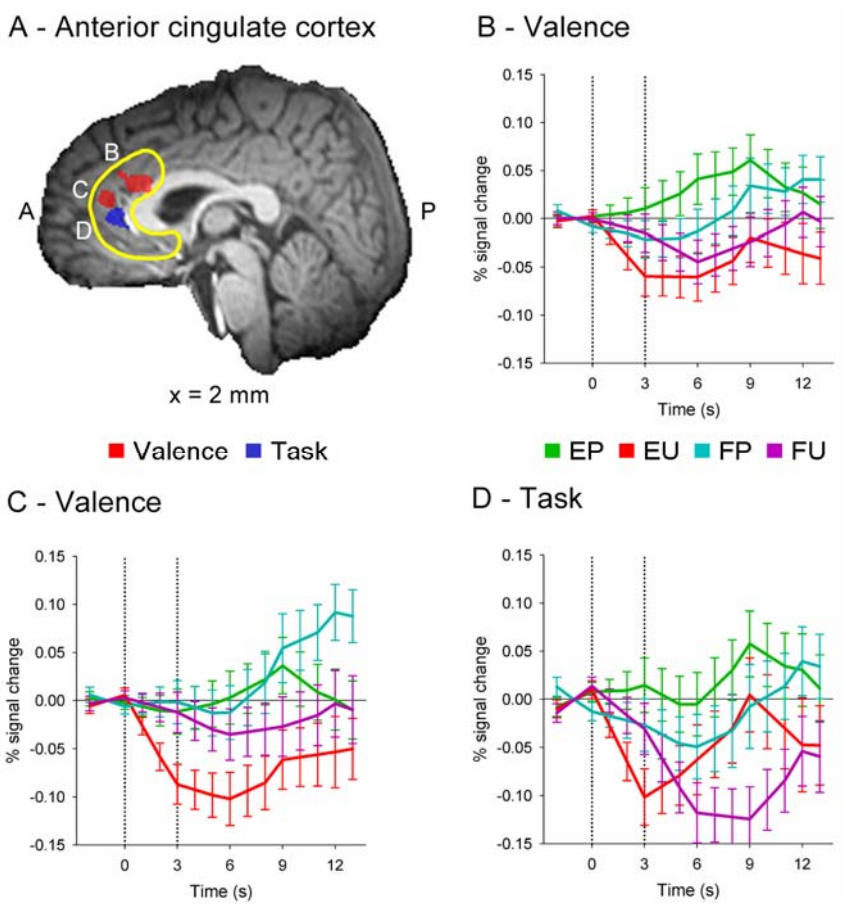

Figure 5-3 Responses in the anterior cingulate cortex. A) Sagittal slice from a single participant indicating regions showing main effects of valence and task at the pACC. Responses are only shown within the *a priori* region of interest (yellow line). A: anterior. P: posterior. X: position of slice in Talairach space. B-D) BOLD responses in the regions illustrated in A. Error bars show standard error. EP: emotion rating on pleasant pictures, EU: emotion rating on unpleasant pictures, FP: frequency rating on pleasant pictures, FU: frequency rating on unpleasant pictures.

CHAPTER 6
DISCUSSION

Summary

The experiments in this dissertation represent an effort to develop a functional magenetic resonance imaging (fMRI) probe for affective disorders. Previous studies of major depressive disorder (MDD) highlighted three brain regions where resting metabolism or responses to simple emotion tasks were altered: the amygdala, orbitofrontal cortex (OFC), and anterior cingulate cortex (ACC) (Davidson et al., 2003;Drevets et al., 1992;Mayberg, 2003;Sheline et al., 2001;Siegle et al., 2002). These regions are posited to be involved respectively in rapid generation (LeDoux, 2000), contextual modification (Rolls, 1999), and explicit appraisal (Lane et al., 1997a) of emotional responses. The working hypothesis of this work is that the amygdala mediates bottom-up (stimulus-driven) emotion processing and that the OFC and ACC mediate top-down (knowledge-based) emotion processing. This hypothesis was investigated under two specific aims:

Aim 1. Assess the validity and reliability of the amygdala response to a face matching paradigm. Face matching elicits activation the amygdala reliably, due, it is thought, to bottom-up processing of the facial features that communicate emotion. In order to separate responses to facial emotion from responses to non-emotional components of face matching, we modified the face matching task to include an intermediate control condition in which neutral faces were matched by identity. The left and right amygdalae responded to both the emotion and the identity matching conditions, and the left amygdala response habituated to emotion matching but not to identity matching. These results suggest that in the face matching task, the amygdala response to emotion is confounded with non-emotional relevance detection involved in matching facial

features. We concluded that amygdala activation in the face matching task is not a specific indicator of bottom-up processing of facial emotion in the amygdala.

Aim 2. Dissociate the neural correlates of top-down and bottom-up emotion processing using a picture rating task. We designed an emotion rating paradigm in which the emotional content of pictures and the type of rating task were varied independently. Bottom up responses were identified mapping the main effect of picture content (pleasant or unpleasant), top-down responses were identified mapping the main effect rating task (emotion rating or frequency rating), and top-down modulation of bottom-up responses was identified by mapping the interaction of picture content and rating task. Bottom-up activation in the amygdala occurred to unpleasant pictures during both emotion rating and frequency rating tasks. The orbitofrontal cortex and insula responses were selective for top-down processing regardless of emotional content. No significant interaction effects were found. The ACC responded to both stimulus content and task demands, and the responses were less specific than those in other regions. Future studies should investigate whether including neutral pictures may elicit interaction effects by varying emotional arousal, and should investigate whether a slower stimulus presentation rate may elicit stronger top-down responses in the ACC. These results showed that an event-related picture rating task can dissociate bottom-up processing in the amygdala from top-down processing in the orbitofrontal cortex and insula. This paradigm allows two brain regions implicated in major depressive disorder to be studied separately and in parallel, and may be used to investigate the effect of illness of bottom-up and top-down emotion processing.

Proof of Concept: Dissociated Responses to Disgust and Arousal

In study one, the disgust study (Chapter 3) we demonstrated dissociated neural correlates of the emotional category disgust and the emotional dimension arousal. We separated disgusting pictures into low arousal (contamination) and high arousal (mutilation) sets, and compared them

with low disgust, high arousal pictures (threat). We showed that disgusting pictures elicited responses in the anterior insula, while emotionally arousing pictures evoked a response in the occipito-temporal cortex. In this study we illustrated the importance of visualizing the changes in BOLD signal that underlie regional responses on the statistical activation map. Comparing signals in this way suggested a relationship between regional responses and emotion ratings that was not obvious from the statistical maps. Investigating this relationship further revealed a quantitative relationship between rating of disgust and the response at the insula, and between ratings of arousal and the response at the occipito-temporal cortex.

A Response at the Amygdala, but not Specific to Emotion

In study one, technical factors prevented detection of responses in the amygdala, a region that has been consistently associated with affective disorders and perception of negative emotional stimuli (Drevets et al., 1992;LeDoux, 2000;Sheline et al., 2001).In study two, we employed a face matching task that had been reported to activate the amygdala reliably, with improved scanning parameters that reduce susceptibility artifact (Chapter 4). We attempted to dissect the emotional component of the face matching task by comparing that task with a similar one in which all faces were neutral. In this way, we hoped to test whether face matching elicited a bottom-up response in the amygdala. Contrary to expectations, activation in the amygdala did not differ when matching emotional faces by expression and when matching neutral faces by identity. A search of the literature suggested that the amygdala responds not only to aversive emotional stimuli, but during any task involving relevance detection, which is the identification of stimuli that may alter the individual's perceived chances of reward (Sander et al., 2003). This notion suggests two interpretations of our results in light of the hypothesized bottom-up role of the amygdala. First, pairs of faces that match are intrinsically relevant, regardless of their emotional content. The amygdala response is therefore bottom-up, being driven by the perceptual

cues presented in facial features, but is not specific to perception of facial emotion. Second, neutral faces usually less relevant than emotional faces, since they convey no social signal of emotion (as suggested by greater amygdala responses to emotional faces than neutral faces (Morris et al., 1996;Whalen et al., 1998b)) and in the identity matching task, the desire to perform a correct match may confer relevance on the matching face [2]. Thus neutral faces acquire temporary relevance, and the associated amygdala response is driven by the demands of the task: a top-down influence. The conclusion drawn from both these interpretations is that the amygdala response during the emotional face matching is contaminated by factors other than bottom-up processing of emotion. However, some features of the responses were selective for emotion. By examining the BOLD responses during separate blocks of each task, we found that the response in the amygdala to emotion matching habituated, a characteristic of responses to emotional stimuli (Breiter et al., 1996;Wright et al., 2001). During identity matching, by contrast, the amygdala response did not habituate. This analysis shows how subtle differences in task paradigm can affect neural responses and blur interpretation.

We chose to investigate the face matching task because it had previously been used in conjunction with a labeling task to demonstrate top-down modulation of bottom-up responses (Hariri et al., 2000). In the previous study, the amygdala response to face matching decreased during face labeling, and was negatively correlated with activity in the right ventrolateral prefrontal cortex. We did not repeat the face labeling experiment because the results of the face matching experiment suggest that the amygdala response to emotion is confounded by task

[2] The control condition – pixel-pattern matching – also required a correct match, but did not activate the amygdala. This may be because of specialization of the amygdala for naturalistic stimuli, especially faces (Leonard et al., 1985); furthermore, parietal activation seen only in control condition suggested pattern matching was performed using spatial processing in the dorsal visual pathway, rather than object processing in the ventral visual pathway (Culham, and Kanwisher, 2001)

demands. We therefore turned to a paradigm in which emotional content and task demands could be controlled separately.

An Event-related Emotion Rating Task Partially Replicates Responses to Block-related Tasks

Previous studies have investigated top-down processing of emotion with rating paradigms. In study three, the emotion rating study (Chapter 5), we designed a task in which the emotional content of presented pictures and the type of rating task were independently controlled. The aims of this task were to dissociate bottom-up responses to picture content from top-down responses during explicit emotion rating, and to demonstrate modulation of bottom-up responses different rating tasks. Participants were shown pleasant and unpleasant pictures, and were either asked to rate how pleasant they found the picture, or how frequently it appeared on television. We varied stimulus content at the event level in order to ensure that bottom-up responses were not confounded with expectation, a top-down factor that may be elicited by blocked stimulus presentation. We also varied task instructions at the event-level to ensure that responses to the two factors were not biased by temporal effects. We employed a factorial analysis in order to identify brain regions responding exclusively to bottom-up or to top-down factors (main effects), and to identify regions in which the two factors interact. Replicating the findings of previous studies, we found a main effect of rating task in the OFC and ACC, and a main effect of stimulus content in the left amygdala. Examination of the BOLD responses in these regions confirmed that the OFC responded to emotion rating regardless of stimulus valence, and that the amygdala responded to unpleasant pictures regardless of the task. Different regions of the ACC exhibited main effects of both top-down and bottom-up factors, but BOLD responses at the ACC only weakly supported the statistical findings. The current study thus succeeded in separating bottom-up and top-down processing in the amygdala and OFC, but failed to replicate previous findings

associating the ACC with top-down processing and showing top-down modulation of responses in the amygdala and insula.

Although previous studies reported that rating pictures modulated the responses in the amygdala and insula (Liberzon et al., 2000;Phan et al., 2004;Taylor et al., 2003), our study failed to replicate these findings. Several features of the design may have contributed to this. First, previous studies compared unpleasant and neutral pictures, which differ in emotional ratings of both valence and arousal, but the current study compared unpleasant and pleasant pictures, which differed in valence but not in arousal. Comparing stimuli with different arousal ratings may be necessary to distinguish bottom-up responses in the insula because this region has been associated with autonomic arousal responses (Critchley et al., 2004). Second, in the current study, the rating task was preceded by a training period. Training may have caused a shift from automatic, general arousal responses mediated by the right amygdala to habitual, conscious responses to specific emotional content mediated by the left amygdala (Gur et al., 2002). Future studies may include neutral pictures in order to compare different levels of emotional arousal, and may investigate how neural responses during training in emotion rating evolve over time.

The current study only found a weak top-down response in the ACC. While the current study used an event-related design, previous studies using block-related designs reported strong top-down responses in the ACC (Lane et al., 1997a;Taylor et al., 2003). The response in the ACC may depend on the establishment of a cognitive set over several consecutive emotion ratings, an effect that is eliminated in event-related designs. The current study also failed to detect top-down modulation of bottom-up responses. The modulating effect may only be elicited by a block-related design or by an event-related design with a slower presentation rate. A previous study investigated the most efficient presentation rate for eliciting bottom-up responses

to emotional stimuli (Serences, 2004). Future studies may investigate the effect of stimulus blocking and presentation rate on top-down responses to emotion rating tasks.

Technical Considerations

Scanning Parameters

In study two, improved scanning parameters facilitated detection of activation in the amygdala. This region is prone to susceptibility artifact, a loss of signal due to the nearby interface between air and tissue at the nasal sinuses. Because scanning parameters that reduce susceptibility artifact may also reduce sensitivity to BOLD signal changes, defining optimal parameters is an ongoing challenge. Several authors have proposed optimized scanning parameters for minimizing susceptibility artifact at the amygdala; however, none of these studies were able to validate their approach using an emotion task because neural responses in the amygdala to emotional stimuli habituate, preventing the comparison of BOLD responses obtained during two successive scans (Chen et al., 2003;Merboldt et al., 2001;Robinson et al., 2004). We nonetheless tested two sets of parameters using emotional stimuli from study one, comparing cubic voxels with side of 3.8 mm or 3.0 mm. Scanning using 3.0 mm voxels produced better images than with 3.8 mm voxels, but BOLD responses in the ventral temporal cortex appeared to lose their sensitivity to different levels of emotional arousal when scanning using 3.0 mm voxels (results not shown). We therefore concluded that scanning with 3.8 mm voxels minimizes susceptibility artifact at the amygdala while maintaining sensitivity to BOLD effects. These parameters achieved coverage of ten out of twelve participants in study two, but in study three coverage of the amygdala was achieved in only ten out of fifteen participants.

An additional challenge arose in the emotion rating study, in which both the amygdala and ACC were regions of interest. Responses in the subgenual ACC (sACC) were discarded in this study due to susceptibility artifact. The interface between air and tissue that causes artifact at the

sACC is nearly perpendicular to the interface near the amygdala. Because the severity of susceptibility artifact depends upon the relationship between the orientations of the air-tissue interface and the plane of scanning, a scanning plane that provides good images of the amygdala is likely to provide poor images of the sACC. Future work should investigate the optimal scanning parameters for imaging both the amygdala and sACC using advanced pulse sequences (such as spiral echo-planar imaging), oral magnetic shim devices (Wilson, and Jezzard, 2003), or smart phantoms designed to mimic BOLD responses in regions of susceptibility artifact.

Connectivity Analysis

The original proposal for this work conceived of the amygdala, OFC, and ACC as a network. For this reason we planned to perform functional connectivity analyses to investigate the interactions between these regions. Functional connectivity analysis estimates communication between brain regions by calculating the correlation between the signals at each region (Buchel et al., 1999). Within-condition interregional covariance analysis (WICA) investigates how this correlation changes as a function of task conditions (He et al., 2003). Our original goal was to repeat the face matching and labeling experiment of Hariri et al. (2000) in order to replicate interactions between the amygdala and prefrontal cortex. Since, however, the results of this study cast doubt upon the emotional significance of the amygdala response, we did not continue to develop this paradigm. In the next experiment, the emotion rating task, we employed an event-related design. Both WICA and the 'psychophysiological interactions' approach used originally in Hariri et al. (2000) investigate the influence of task on functional connectivity by calculating BOLD signal correlations between brain regions during different task blocks (Friston et al., 1997). In our rapid, event-related design, the responses to different event types were commingled, and could not be analyzed in this way. Further development of novel analysis techniques may allow functional connectivity analysis within event-related designs.

Current Trends in Functional Imaging of Major Depressive Disorder

Recent functional neuroimaging studies of MDD have focused on three features of the illness: emotional bias, impaired regulation of emotion, and impaired concentration (Leppanen, 2006). Patients with depression pay more attention to negative stimuli and less attention to positive stimuli, whereas healthy controls tend to have the opposite bias. This shift in bias may be revealed by behavioral measures of attention, or by identifying brain regions that reverse their responses to positive and negative stimuli in patients with MDD. Impaired emotional regulation has so far been investigated only indirectly, by looking at altered patterns of cortico-limbic connectivity in patients compared with controls. Impaired concentration has been investigated with memory and attention paradigms.

Emotional Bias

Several imaging studies have shown that in MDD the subcortical and cortical responses to positive and negative stimuli reverse their sign. The putamen and fusiform gyrus showed greater activation to happy faces than sad faces in controls, but showed greater activation to sad faces than happy faces in patients (Surguladze et al., 2005). Similarly, responses to sad faces at the ventral striatum and amygdala were elevated in patients with depression (Fu et al., 2004). Treatment with antidepressant medication reduced these limbic responses, and increased the response in the lateral prefrontal cortex. A corresponding reversal was observed in the ventromedial prefrontal cortex, which responded to happy mood induction in controls, and to sad mood induction in patients (Keedwell et al., 2005). These findings show that biased responses to emotional stimuli may be elicited in both cortical and subcortical regions. However, the passive viewing paradigms used to elicit subcortical responses and the mood induction paradigm used to elicit cortical responses are not directly comparable. Future studies may investigate subcortical and cortical biases side by side using tasks designed to separate top-down and bottom-up

emotion processing, such as the emotion rating task presented in Chapter 5. As already discussed, this task must be improved in order to elicit reliable responses in ventromedial prefrontal regions. Following such an improvement, studies of this kind with a sufficiently large patient group may reveal heterogeneity in neural response bias, with some patients exhibiting bias in cortical regions, and others exhibiting bias in subcortical regions. This approach may be used to investigate the neural basis for heterogeneity in symptom profiles.

The effect of emotional bias may be revealed behaviorally by measuring its effect on memory. Patients with depression recall a larger proportion of negative words and a smaller proportion of positive words than healthy controls. Electroencephalographic evidence suggests that this bias in recall is mediated by attentional bias during the encoding of stimuli (Leppanen, 2006). The neural correlates of encoding were investigated using event-related fMRI, comparing responses to faces that were later recalled with those that were not. Successfully recalled negative faces evoked greater responses in the left amygdala in adolescents with MDD compared with controls (Roberson-Nay et al., 2006). A similar effect was seen in adults with remitted depression, but only after mood challenge (Ramel et al., 2006). This implies that bias towards negative stimuli is enhanced following an insult to emotional regulation. By using a behavioral measure of emotional bias to guide the analysis of functional imaging data, these experiments provide evidence linking increased amygdala activity in MDD with increased attention to and encoding of negative emotional stimuli.

Behavioral measures of emotional bias may also be obtained using a dot probe task or by measuring facial expression recognition thresholds. In dot-probe tasks, two faces are displayed, followed by a dot in the location of one of the two faces. The participant must respond as quickly as possible to the location of the dot. Patients with MDD respond faster when the dot is displayed

in the location previously occupied by a sad face, and several studies have confirmed that this effect is specific to MDD, and is not seen in patients with general anxiety disorder or social phobia (Leppanen, 2006). A dot probe task with fearful faces was used in an fMRI study of healthy individuals (Pourtois, and Vuilleumier, 2006). This study compared valid trials, in which the fearful face was shown in the same location as the subsequent dot, with invalid trials, in which the fearful face was shown on the opposite side as the dot. During valid trials, response increased in the occipital cortex. During invalid trials, responses decreased in the parietal cortex and increased in the orbitofrontal cortex. These results were proposed to reflect the attentional draw of fearful faces during valid trials, and the increased cost of disengaging attention from fearful faces during invalid trials. Because the dot probe task provides a behavioral measure of the effect of emotion on attention, it represents a more powerful paradigm than passive face viewing for the investigation of affective disorders.

Facial expression recognition thresholds are measured by having participants judge pictures of faces that have been morphed to display varying degrees of emotion. Patients with MDD and healthy individuals who report anhedonia fail to recognize happy expressions that are recognized by healthy controls (Leppanen, 2006). While morphed faces have been used in fMRI studies of MDD (Fu et al., 2004;Surguladze et al., 2005), recognition thresholds have not yet been used as a behavioral covariate in fMRI analysis. Patients with MDD may experience a greater implicit attentional draw toward sad faces in the dot-probe task, and fail to explicitly recognize happy faces in the recognition threshold tasks. These results imply that bias toward sad faces and bias against happy faces may be mediated respectively by bottom-up and top-down systems. Future imaging studies of patients performing these tasks should address this hypothesis.

Emotion Regulation

Recent studies of patients with MDD have investigated emotional regulation indirectly, by measuring correlation between cortical and limbic responses. These interactions were investigated using a new method that measures low frequency fluctuations in the blood oxygenation-level dependent signal (LFBF) (Anand et al., 2005a). In patients with MDD compared with healthy controls, negative emotional pictures evoked increased responses in the ACC, amygdala, ventral striatum, and medial thalamus. However, correlations in LFBF between ACC and limbic regions were reduced. These correlations were increased in patients following antidepressant treatment, suggesting that medication restored regulation (Anand et al., 2005b). Impairment of regulation may also predict patients' responses to cognitive behavioral therapy. Successful therapy was predicted by low responses at the subgenual ACC and high responses at the amygdala during a task in which participants briefly saw emotional words and then attempted to sustain their emotional responses (Siegle et al., 2006). This pattern of activity was proposed to represent decreased regulation by the sACC and increased emotional reactivity at the amygdala, suggesting that patients with impaired regulation benefit most from cognitive behavioral therapy. The latter experiment may be improved by measuring correlations between the subgenual ACC and amygdala. The former experiments may be improved using a paradigm with several task condition that are designed to vary the degree of emotional regulation, and to thus potentially alter cortico-limbic connectivity. As already discussed, this effect was demonstrated by comparing emotional face matching with face labeling (Hariri et al., 2000). The correlation between BOLD signal in the amygdala and in the right ventral PFC reversed its sign between the two conditions. Increased prefrontal and decreased amygdala responses during labeling were interpreted as representing emotion regulation. However, our investigation of the face matching task suggested that the amygdala response to emotion was confounded with non-emotional

recognition of matching perceptual features. Other studies have investigated emotion regulation in healthy individuals, but none has repeated the connectivity approach of Hariri et al. (2000). A review of studies of the cognitive control of emotion proposed two systems for emotional regulation in the prefrontal cortex, a dorsal indirect system, and a ventral direct system (Ochsner, and Gross, 2005). The former was associated with tasks in which emotions were altered by reappraisal, and the second with tasks in which emotions were altered by extinction or reversal. The same regions associated with indirect regulation also showed greater activation in patients than controls performing cognitive tasks, as discussed below.

Cognitive Tasks

MDD is characterized by impaired concentration; therefore patients may also have altered neural responses to non-emotional, cognitively-demanding tasks. Several authors have studied the neural correlates of attention and working memory in patients with MDD. The Stroop task tests attention by requiring participants to name the ink color of a word that names a different color. This requires them to pay attention to one aspect of a stimulus and ignore another. The n-back task exercises working memory by presenting a stream of characters, and asking participants to respond to stimuli that match a previously presented stimulus. This requires them to hold in memory a sequence of characters, and to shift and update that sequence with every new character. Patients with MDD exhibit increased responses to both the Stroop and n-back tasks in the ACC and the lateral and dorsolateral PFC (Harvey et al., 2005;Matsuo et al., 2006;Wagner et al., 2006). Conversely, load-dependent responses to an n-back task were decreased in patients at the medial orbitofrontal cortex and rostral ACC (Rose et al., 2006). Despite altered neural responses, patients performed as well as controls on all tasks. Increased responses in dorsolateral PFC were interpreted as representing greater effort required to achieve equal performance. The results may reflect an effect of mood state on the neural responses

during cognitive task performance. To date, no functional imaging studies of patients with MDD have investigated whether responses during cognitive tasks may be modulated by the addition of emotional stimuli. However, such studies in healthy volunteers have supported the idea that cognitive and emotional circuits compete, particularly in the anterior cingulate cortex. Because this region is being seen as increasingly important in affective disorders, combining cognitive tasks with emotional stimuli may prove a promising approach for future studies.

Future Direction: the Anterior Cingulate Cortex and the Interaction of Emotion and Cognition

Mayberg's model of major depressive disorder emphasizes the competition between a dorsal, cognitive or sensorimotor circuit and a ventral, emotional or visceral / autonomic circuit (Mayberg, 2003). This competition between cognitive and emotional circuits was also noted in an early review of PET studies. Tasks involving visual discrimination and working memory activated the dorsolateral PFC and dorsal ACC, but deactivated the ventromedial PFC and ventral ACC (Drevets, and Raichle, 1998b). The opposite pattern was seen for tasks involving mood induction, emotional stimulus viewing, and symptom provocation in patients with affective disorders: these deactivated the dorsolateral PFC and dorsal ACC, but activated the ventromedial PFC, ventral ACC, and – in tasks involving visual stimuli – the amygdala. A later review of fMRI studies confirmed opposing patterns of responses in the dorsal and ventral ACC to cognitive and emotional tasks, suggesting that different regions of the ACC perform similar functions in cognitive and emotional contexts (Bush et al., 2000). The ventral ACC may be further divided into pregenual and subgenual subdivisions. Resting metabolism in the pregenual ACC may predict recovery from depression, and resting metabolism in the subgenual ACC is elevated in patients. The importance of the subgenual ACC in depression was highlighted by a recent pilot study of deep brain stimulation (Mayberg et al., 2005). Six patients with multiple

drug-resistant depression had electrodes implanted near the subgenual ACC. Electrical stimulation was accompanied by improved symptoms in four out of six patients. Functional imaging paradigms designed to activate the subdivisions of the ACC selectively may prove informative in the study of major depressive disorder.

The dorsal and ventral divisions of the ACC may mediate conflict monitoring in cognitive and emotional domains (Bush et al., 2000). This was suggested by studies dissociating dorsal and ventral ACC responses to cognitive and emotional Stroop tasks (Bush et al., 1998;Whalen et al., 1998a). In Stroop tasks, conflicting stimuli are used to interfere with responses to a task; in this case, participants were instructed to count the number of words on the screen. Cognitive conflict was elicited by displaying distracting number words, for example, the number "two" displayed four times, which prompted two conflicting responses within the task set. Emotional conflict was elicited by displaying emotional words, with the assumption that the resulting emotional response would interfere with counting. Cognitive conflict activated the dorsal ACC (Bush et al., 1998), whereas emotional conflict activated the ventral ACC (Whalen et al., 1998a). Cognitive theorists debate the extent to which cognitive and emotional conflict are comparable, but agree that both involve the diversion of attentional resources from correct response selection (Algom et al., 2004;Chajut et al., 2005;Dalgleish, 2005). In cognitive conflict this occurs within the task-related response set, whereas in emotional conflict attention is drawn outside the task-related response set. A mechanism for regulation of attention during cognitive conflict was proposed in the conflict hypothesis, which states that detection of conflict results in increased cognitive control (Cohen et al., 1990;Cohen et al., 2000). Conflict monitoring and cognitive control were associated respectively with the dorsal ACC and dorsolateral PFC. The dorsal ACC was activated selectively by conflicting Stroop trials, whereas the dorsolateral PFC was activated

selectively by instructions announcing difficult trials (which may elicit anticipatory cognitive control) (MacDonald, III et al., 2000). Furthermore, trial-by-trial analysis revealed that dorsal ACC activation to conflicting Stroop trials was followed in subsequent trials by increased dorsolateral PFC activation and slower response times (indicating increased control) (Kerns et al., 2004). Extending this model of cognitive conflict to the emotional domain may provide a useful framework for working hypotheses about the role of the ventral ACC in emotion processing, and its functional interactions with other regions.

The ventral ACC consists of two subdivisions, which may each play a distinct role in conflict monitoring, and which may each have distinct functional relationships with other brain regions. The pregenual ACC and subgenual ACC lie anterior and inferior to the genu of the corpus callosum. The subgenual ACC may detect emotional conflict in the visceral / autonomic circuit and enhance processing in this circuit while decreasing processing in the sensorimotor circuit. This region was associated with symptoms of depression and in healthy individuals with sad mood induction (Mayberg et al., 1997;Vogt, 2005). The role of the subgenual ACC in the withdrawal component of sad mood is supported by evidence in monkeys that the subgenual ACC sends projections to the ventrolateral region of the periaqueductal gray area, allowing it to coordinate a quiescent stance (Ongur, and Price, 2000), and by evidence that neurons in the subgenual ACC fire as monkeys prepare to sleep (Rolls et al., 2003). The pregenual ACC may detect conflict at a higher level, between the sensorimotor and visceral / autonomic circuits, and recruit emotional control, allowing engagement in sensorimotor activity despite concurrent emotional conflict. This region was shown to predict recovery in patients with depression and in healthy individuals responded to happy mood induction (Mayberg et al., 1997;Vogt, 2005). The pregenual ACC responded during successful performance of a Stroop task with emotional

distractors (Whalen et al., 1998a) and responded selectively to tasks involving symbolic representation of emotion (such as placebo and expectancy tasks) (Eisenberger, and Lieberman, 2004). The role of the pregenual ACC in engagement is supported by evidence in monkeys that the pregenual ACC sends projections to the lateral region of the periaqueductal gray, allowing it to coordinate a confrontational or engaged stance (Ongur, and Price, 2000). Studies of cognitive conflict described a functional relationship between the dorsal ACC and the dorsolateral PFC (Kerns et al., 2004;MacDonald, III et al., 2000). The pregenual and subgenual ACC may have parallel functional relationships. A recent study in humans used diffusion-weighted imaging to trace fibers from the ACC, showing specific connections between the subgenual ACC and amygdala, and between the pregenual ACC and ventromedial PFC. The amygdala has been associated with both the generation of visceral / autonomic responses (Critchley et al., 2002), which may signal emotional conflict, and with the direction of attention towards emotionally salient stimuli (Adolphs et al., 2005;Sander et al., 2003), which may be a component of withdrawal from sensorimotor activity following detection of emotional conflict. The ventromedial PFC has been associated with emotional control (Ochsner, and Gross, 2005), and may be recruited via connections from the pregenual ACC after this region detects conflict between cognitive and emotional circuits. Taken together, these findings suggest that activation of the subgenual ACC may occur when emotional stimuli elicit withdrawal from a cognitive task, and that activation of the pregenual ACC may occur when emotional stimuli are successfully ignored, allowing continued engagement with a cognitive task. Functional imaging paradigms involving cognitive tasks with emotional distractors may be used to test these predictions, and to investigate functional relationships between the subgenual ACC and amygdala and between the pregenual ACC and ventromedial PFC.

Designing a task paradigm in which emotional stimuli impair the participant's involvement in a cognitive task may be challenging, but may advance our understanding of the role of the ACC in depression and in normal emotion processing. The emotional Stroop task above activated the pregenual ACC, but not the subgenual ACC (Whalen et al., 1998a). In this task, emotional words did not affect task performance. Therefore, one challenge for future studies of this type will be to elicit emotional responses that are sufficiently strong to impair cognitive task performance. Furthermore, emotional conflict should be elicited at several levels: increasing conflict should result in decreasing task performance. Emotional ratings of stimuli at each level and measures of task performance could be used to define individual "engagement thresholds", enabling parametric analysis of the neural correlates of disengagement. A second challenge for these studies will be to investigate functional relationships between the subgenual ACC and amygdala, and between the pregenual ACC and ventromedial PFC. This may be pursued first using functional connectivity analysis, and second using trial-by-trial analysis to dissociate conflict detection from subsequent recruitment of control, as was done for cognitive conflict (Kerns et al., 2004). These approaches may elucidate normal emotion processing by testing whether the subdivisions of the ACC play specific roles in mediating emotional influences on cognitive task performance. Testing functional relationships of the ventral subdivisions of the ACC will test whether the functional interactions seen in cognitive conflict can be extended to emotional conflict. These approaches may also elucidate the neural bases for depression by providing a specific probe for activity in the subgenual ACC, which is implicated in depressed mood, and in the pregenual ACC, which is proposed to mediate recovery from depression. Dissociating emotional conflict monitoring from emotional control may reveal whether depressed mood is mediated by hypersensitivity to conflict, or by impaired control. Future

studies may continue to build more refined models of emotion processing, and thus may pave the way for improved understanding of the neural bases of affective disorders. The functional neuroimaging paradigms developed in these studies may eventually be used to guide the choice of conventional treatments, and to guide the development of novel treatments such as deep brain stimulation.

APPENDIX
IAPS PICTURES CODES

Table A-1 Contamination pictures.

Code	Description
1270	Roach
1274	Roaches
1275	Roaches
1280	Rat
1945	Turtle
2720	Urinating
2730	NativeBoy
7360	FliesOnPie
7380	RoachOnPizza
9005	HIV Tattoo
9006	HIV Tattoo
9008	Needle
9090	Exhaust
9280	Smoke
9290	Garbage
9300	Dirty
9320	Vomit
9330	Garbage
9340	Garbage
9373	Garbage
9390	Dishes
9560	Duck in oil
9561	Sick kitty
9700	Workers-trash
9830	Cigarettes

Table A-2 Mutilation pictures.

Code	Description
3000	Mutilation
3010	Mutilation
3010	Mutilation
3015	Accident
3030	Mutilation
3051	Mutilation
3053	Burn victim
3060	Mutilation
3061	Mutilation
3062	Mutilation
3063	Mutilation
3064	Mutilation
3071	Mutilation
3080	Mutilation
3100	Burn victim
3102	Burn victim
3110	Burn victim
3120	Dead body
3130	Mutilation
3140	Dead body
3150	Mutilation
3168	Mutilation
3170	Baby tumor
3181	BatteredFem
3250	OpenChest
3261	Tumor
3266	Injury
3400	Severed hand
9253	Mutilation
9265	Hung man
9405	Sliced hand
9430	Burial
9433	Dead man
9490	Corpse

Table A-3 Threat pictures

Code	Description
1050	Snake
1051	Snake
1052	Snake
1120	Snake
1230	Spider
1300	Pit Bull
1931	Shark
2682	Police
3500	Attack
3530	Attack
6230	Aimed Gun
6242	Gang
6243	AimedGun
6244	AimedGun
6250	AimedGun
6260	AimedGun
6300	Knife
6314	Attack
6350	Attack
6510	Attack

Table A-4 Neutral pictures

Code	Description	Code	Description	Code	Description
1450	Gannet	5740	Plant	7190	Clock
1510	Dog	5750	Nature	7205	Scarves
1601	Giraffes	5800	Leaves	7207	Beads
1603	Butterfly	5831	Seagulls	7211	Clock
1670	Cow	5875	Bicyclist	7217	Clothes rack
1670	Cow	5900	Desert	7224	File cabinets
1812	Elephants	6150	Outlet	7233	Plate
1999	Mickey	7000	Rolling Pin	7234	Ironing board
2000	Adult	7002	Towel	7235	Chair
2058	Baby	7004	Spoon	7285	Tomatoes
2312	Mother	7006	Bowl	7325	Watermelon
2331	Chef	7009	Mug	7490	Window
2383	Secretary	7010	Basket	7491	Building
2389	Teens	7020	Fan	7495	Store
2487	Musician	7025	Stool	7496	Street
2514	Woman	7030	Iron	7500	Building
2575	Propeller	7031	Shoes	7501	City
2580	Chess	7034	Hammer	7545	Ocean
2655	Child	7035	Mug	7550	Office
2791	Balloons	7040	Dust pan	7560	Freeway
2840	Chess	7050	Hair dryer	7595	Traffic
2870	Teenager	7060	Trash Can	7600	Dragon
2880	Shadow	7080	Fork	7620	Jet
5020	Flower	7090	Book	7705	Cabinet
5120	Pine needles	7095	Headlight	7710	Bed
5200	Flowers	7096	Car	7900	Violin
5220	Nature	7100	Fire hydrant	7950	Tissue
5300	Galaxy	7110	Hammer	8160	RockClimber
5390	Boat	7130	Truck	8161	Hang glider
5410	Violinist	7140	Bus	8162	HotAirBalloon
5510	Mushroom	7150	Umbrella		
5534	Mushrooms	7160	Fabric		
5600	Mountains	7170	Light Bulb		
5720	Farmland	7175	Lamp		

Table A-5 Emotion rating pictures

Pleasant		Unpleasant	
Code	Description	Code	Description
1670	Cow	1050	Snake
1726	Jaguars	1052	Snake
1812	Elephants	1101	Snake
2391	Boy	1120	Snake
4533	AttractiveMan	1280	Rat
5260	Waterfall	1300	Pit Bull
5480	Fireworks	2440	NeutGirl
5750	Nature	2691	Riot
5780	Nature	2870	Teenager
5910	Fireworks	4621	Harassment
7080	Fork	5531	Mushroom
7270	IceCream	6000	Prison
8021	Skier	7006	Bowl
8186	Skydivers	9102	Heroin
8340	Plane	9600	Ship

Table A-6 Frequency rating pictures

Pleasant		Unpleasant	
Code	Description	Code	Description
1313	Frog	2141	GrievingFem
1340	Women	2312	Mother
1920	Porpoise	2690	Terrorist
1999	Mickey	2752	Alcoholic
2160	Father	2880	Shadow
2170	Mother	6200	AimedGun
2579	Propeller	6243	AimedGun
4274	AttractiveFem	6315	Attack
4689	EroticCouple	7037	Mug
5270	Nature	7090	Book
5623	Windsurfers	7110	Hammer
5991	Sky	9110	Puddle
6250.2	IceCream	9182	Horses
8178	Sailboat	9190	Woman
8370	Rafting	9415	Handicapped

LIST OF REFERENCES

Adolphs,R. (2002). Neural systems for recognizing emotion. Current Opinion in Neurobiology *12*, 169-177.

Adolphs,R., Gosselin,F., Buchanan,T.W., Tranel,D., Schyns,P., and Damasio,A.R. (2005). A mechanism for impaired fear recognition after amygdala damage. Nature *433*, 68-72.

Adolphs,R., Tranel,D., and Damasio,A.R. (2003). Dissociable neural systems for recognizing emotions. Brain and Cognition *52*, 61-69.

Adolphs,R., Tranel,D., Damasio,H., and Damasio,A. (1994). Impaired recognition of emotion in facial expressions following bilateral damage to the human amygdala. Nature *372*, 669-672.

Aggleton,J.P., and Saunders,R.C. (2000). The amygdala - what's hapenned in the last decade? In The Amygdala, J. P. Aggleton, ed. (Oxford: Oxford University Press), pp. 1-30.

Aguirre,G.K., Zarahn,E., and D'Esposito,M. (1998). The variability of human, BOLD hemodynamic responses. Neuroimage *8*, 360-369.

Algom,D., Chajut,E., and Lev,S. (2004). A rational look at the emotional Stroop phenomenon: a generic slowdown, not a stroop effect. Journal of Experimental Psychology: General *133*, 323-338.

Anand,A., Li,Y., Wang,Y., Wu,J., Gao,S., Bukhari,L., Mathews,V.P., Kalnin,A., and Lowe,M.J. (2005a). Activity and connectivity of brain mood regulating circuit in depression: a functional magnetic resonance study. Biological Psychiatry *57*, 1079-1088.

Anand,A., Li,Y., Wang,Y., Wu,J., Gao,S., Bukhari,L., Mathews,V.P., Kalnin,A., and Lowe,M.J. (2005b). Antidepressant effect on connectivity of the mood-regulating circuit: an FMRI study. Neuropsychopharmacology *30*, 1334-1344.

Anderson,A.K., Christoff,K., Panitz,D., De Rosa,E., and Gabrieli,J.D. (2003). Neural correlates of the automatic processing of threat facial signals. Journal of Neuroscience *23*, 5627-5633.

Bannon,M.J., and Roth,R.H. (1983). Pharmacology of mesocortical dopamine neurons. Pharmacol.Rev. *35*, 53-68.

Bechara,A., Damasio,A.R., Damasio,H., and Anderson,S.W. (1994). Insensitivity to future consequences following damage to human prefrontal cortex. Cognition *50*, 7-15.

Blamire,A.M., Ogawa,S., Ugurbil,K., Rothman,D., McCarthy,G., Ellermann,J.M., Hyder,F., Rattner,Z., and Shulman,R.G. (1992). Dynamic mapping of the human visual cortex by high-speed magnetic resonance imaging. Proceedings of the National Academy of Sciences of the United States of America *89*, 11069-11073.

Boynton,G.M., Engel,S.A., Glover,G.H., and Heeger,D.J. (1996). Linear systems analysis of functional magnetic resonance imaging in human V1. Journal of Neuroscience *16*, 4207-4221.

Breiter,H.C., Etcoff,N.L., Whalen,P.J., Kennedy,W.A., Rauch,S.L., Buckner,R.L., Strauss,M.M., Hyman,S.E., and Rosen,B.R. (1996). Response and habituation of the human amygdala during visual processing of facial expression. Neuron *17*, 875-887.

Brierley,B., Shaw,P., and David,A.S. (2002). The human amygdala: a systematic review and meta-analysis of volumetric magnetic resonance imaging. Brain Research.Brain Research Reviews *39*, 84-105.

Broks,P., Young,A.W., Maratos,E.J., Coffey,P.J., Calder,A.J., Isaac,C.L., Mayes,A.R., Hodges,J.R., Montaldi,D., Cezayirli,E., Roberts,N., and Hadley,D. (1998). Face processing impairments after encephalitis: amygdala damage and recognition of fear. Neuropsychologia *36*, 59-70.

Buccino,G., Binkofski,F., and Riggio,L. (2004). The mirror neuron system and action recognition. Brain and Language *89*, 370-376.

Buchel,C., Coull,J.T., and Friston,K.J. (1999). The predictive value of changes in effective connectivity for human learning. Science *283*, 1538-1541.

Buchel,C., Holmes,A.P., Rees,G., and Friston,K.J. (1998). Characterizing stimulus-response functions using nonlinear regressors in parametric fMRI experiments. Neuroimage *8*, 140-148.

Burock,M.A., Buckner,R.L., Woldorff,M.G., Rosen,B.R., and Dale,A.M. (1998). Randomized event-related experimental designs allow for extremely rapid presentation rates using functional MRI. Neuroreport *9*, 3735-3739.

Bush,G., Luu,P., and Posner,M.I. (2000). Cognitive and emotional influences in anterior cingulate cortex. Trends in Cognitive Science *4*, 215-222.

Bush,G., Whalen,P.J., Rosen,B.R., Jenike,M.A., McInerney,S.C., and Rauch,S.L. (1998). The counting Stroop: an interference task specialized for functional neuroimaging--validation study with functional MRI. Human Brain Mapping *6*, 270-282.

Calder,A.J., Keane,J., Manes,F., Antoun,N., and Young,A.W. (2000). Impaired recognition and experience of disgust following brain injury. Nature Neuroscience *3*, 1077-1078.

Center for the Study of Emotion and Attention [CSEA-NIMH] (2001). The international affective picture system: Digitized photographs. (Gainesville, FL: The Center for Research in Psychophysiology, University of Florida).

Chajut,E., Lev,S., and Algom,D. (2005). Vicissitudes of a misnomer: reply to Dalgleish (2005). Journal of Experimental Psychology: General *134*, 592-595.

Chao,L.L., Martin,A., and Haxby,J.V. (1999). Are face-responsive regions selective only for faces? Neuroreport *10*, 2945-2950.

Chen,N.K., Dickey,C.C., Yoo,S.S., Guttmann,C.R., and Panych,L.P. (2003). Selection of voxel size and slice orientation for fMRI in the presence of susceptibility field gradients: application to imaging of the amygdala. Neuroimage *19*, 817-825.

Cohen,J.D., Botvinick,M., and Carter,C.S. (2000). Anterior cingulate and prefrontal cortex: who's in control? Nature Neuroscience *3*, 421-423.

Cohen,J.D., Dunbar,K., and McClelland,J.L. (1990). On the control of automatic processes: a parallel distributed processing account of the Stroop effect. Psychological Review *97*, 332-361.

Cohen,R.A., Kaplan,R.F., Zuffante,P., Moser,D.J., Jenkins,M.A., Salloway,S., and Wilkinson,H. (1999). Alteration of intention and self-initiated action associated with bilateral anterior cingulotomy. Journal of Neuropsychiatry and Clinical Neurosciences *11*, 444-453.

Critchley,H., Daly,E., Phillips,M., Brammer,M., Bullmore,E., Williams,S., Van Amelsvoort,T., Robertson,D., David,A., and Murphy,D. (2000). Explicit and implicit neural mechanisms for processing of social information from facial expressions: a functional magnetic resonance imaging study. Human Brain Mapping *9*, 93-105.

Critchley,H.D. (2005). Neural mechanisms of autonomic, affective, and cognitive integration. Journal of Comparative Neurology *493*, 154-166.

Critchley,H.D., Mathias,C.J., and Dolan,R.J. (2002). Fear conditioning in humans: the influence of awareness and autonomic arousal on functional neuroanatomy. Neuron *33*, 653-663.

Critchley,H.D., Mathias,C.J., Josephs,O., O'Doherty,J., Zanini,S., Dewar,B.K., Cipolotti,L., Shallice,T., and Dolan,R.J. (2003). Human cingulate cortex and autonomic control: converging neuroimaging and clinical evidence. Brain *126*, 2139-2152.

Critchley,H.D., Wiens,S., Rotshtein,P., Ohman,A., and Dolan,R.J. (2004). Neural systems supporting interoceptive awareness. Nature Neuroscience *7*, 189-195.

Culham,J.C., and Kanwisher,N.G. (2001). Neuroimaging of cognitive functions in human parietal cortex. Current Opinion in Neurobiology *11*, 157-163.

Cunningham,W.A., Raye,C.L., and Johnson,M.K. (2004). Implicit and explicit evaluation: FMRI correlates of valence, emotional intensity, and control in the processing of attitudes. Journal of Cognitive Neuroscience *16*, 1717-1729.

Dale,A.M., and Buckner,R.L. (1997). Selective Averaging of Rapidly Presented Individual Trials Using fMRI. Human Brain Mapping *5*, 329-340.

Dalgleish,T. (2005). Putting some feeling into it--the conceptual and empirical relationships between the classic and emotional Stroop tasks: comment on Algom, Chajut, and Lev (2004). Journal of Experimental Psychology: General *134*, 585-591.

Damasio,A., and Van Hoesen,G.W. (1983). Emotional disturbances associated with focal lesions of the limbic frontal lobe. In Neuropsychology in Human Emotion, K. M. Heilman and P. Satz, eds. (New York: Guilford Press), pp. 85-110.

Davidson,R.J., and Irwin,W. (1999). The functional neuroanatomy of emotion and affective style. Trends in Cognitive Science *3*, 11-21.

Davidson,R.J., Irwin,W., Anderle,M.J., and Kalin,N.H. (2003). The neural substrates of affective processing in depressed patients treated with venlafaxine. American Journal of Psychiatry *160*, 64-75.

Dehaene,S., Molko,N., Cohen,L., and Wilson,A.J. (2004). Arithmetic and the brain. Current Opinion in Neurobiology *14*, 218-224.

Drevets,W.C. (1998). Functional neuroimaging studies of depression: the anatomy of melancholia. Annual Review of Medicine *49*, 341-361.

Drevets,W.C., and Raichle,M.E. (1998a). Reciprocal Suppression of Regional Cerebral Blood Flow during Emotional versus Higher Cognitive Processes: Implications for Interactions between Emotion and Cognition. Cognition and Emotion *12*, 353-385.

Drevets,W.C., and Raichle,M.E. (1998b). Reciprocal suppression of regional cerebral blood flow during emotional versus higher cognitive processes: implications for interactions between emotion and cognition. Cognition and Emotion *12*, 353-385.

Drevets,W.C., Videen,T.O., Price,J.L., Preskorn,S.H., Carmichael,S.T., and Raichle,M.E. (1992). A functional anatomical study of unipolar depression. Journal of Neuroscience *12*, 3628-3641.

Eisenberger,N.I., and Lieberman,M.D. (2004). Why rejection hurts: a common neural alarm system for physical and social pain. Trends in Cognitive Sciences *8*, 294-300.

Ekman,P. (1982). Emotion in the human face. (Cambridge [Cambridgeshire]: Cambridge University Press).

Ekman,P., and Friesen,W.V. (1976). Pictures of facial affect. (Palo Alto, Ca.: Consulting Psychologists Press).

Ellsworth,P.C. (1994). James,William and emotion - is a century of fame worth a century of misunderstanding? Psychological Review *101*, 222-229.

Eysenck,W., and Keane,M.T. (2000). Cognitive psychology. (Philadelphia: Taylor and Francis).

Friston,K.J., Buechel,C., Fink,G.R., Morris,J., Rolls,E., and Dolan,R.J. (1997). Psychophysiological and modulatory interactions in neuroimaging. Neuroimage *6*, 218-229.

Friston,K.J., Josephs,O., Rees,G., and Turner,R. (1998). Nonlinear event-related responses in fMRI. Magnetic Resonance in Medicine *39*, 41-52.

Friston,K.J., Price,C.J., Fletcher,P., Moore,C., Frackowiak,R.S., and Dolan,R.J. (1996). The trouble with cognitive subtraction. Neuroimage *4*, 97-104.

Fu,C.H., Williams,S.C., Cleare,A.J., Brammer,M.J., Walsh,N.D., Kim,J., Andrew,C.M., Pich,E.M., Williams,P.M., Reed,L.J., Mitterschiffthaler,M.T., Suckling,J., and Bullmore,E.T. (2004). Attenuation of the neural response to sad faces in major depression by antidepressant treatment: a prospective, event-related functional magnetic resonance imaging study. Archives of General Psychiatry *61*, 877-889.

Glascher,J., and Adolphs,R. (2003). Processing of the arousal of subliminal and supraliminal emotional stimuli by the human amygdala. Journal of Neuroscience *23*, 10274-10282.

Goldapple,K., Segal,Z., and Garson,C. (2002). Effects of cognitive behavioral therapy on brain glucose metabolism in patients with major depression. Biological Psychiatry *51*, 66S.

Goldapple,K., Segal,Z., Garson,C., Lau,M., Bieling,P., Kennedy,S., and Mayberg,H. (2004). Modulation of cortical-limbic pathways in major depression: treatment-specific effects of cognitive behavior therapy. Archives of General Psychiatry *61*, 34-41.

Gorno-Tempini,M.L., Pradelli,S., Serafini,M., Pagnoni,G., Baraldi,P., Porro,C., Nicoletti,R., Umita,C., and Nichelli,P. (2001). Explicit and incidental facial expression processing: an fMRI study. Neuroimage *14*, 465-473.

Gottfried,J.A., O'Doherty,J., and Dolan,R.J. (2003). Encoding predictive reward value in human amygdala and orbitofrontal cortex. Science *301*, 1104-1107.

Gur,R.C., Erwin,R.J., Gur,R.E., Zwil,A.S., Heimberg,C., and Kraemer,H.C. (1992). Facial emotion discrimination: II. Behavioral findings in depression. Psychiatry Research *42*, 241-251.

Gur,R.C., Schroeder,L., Turner,T., McGrath,C., Chan,R.M., Turetsky,B.I., Alsop,D., Maldjian,J., and Gur,R.E. (2002). Brain activation during facial emotion processing. Neuroimage *16*, 651-662.

Haidt,J., McCauley,C., and Rozin,P. (1994). Individual-differences in sensitivity to disgust - a scale sampling 7 domains of disgust elicitors. Personality and Individual Differences *16*, 701-713.

Hamzei,F., Rijntjes,M., Dettmers,C., Glauche,V., Weiller,C., and Buchel,C. (2003). The human action recognition system and its relationship to Broca's area: an fMRI study. Neuroimage *19*, 637-644.

Hariri,A.R., Bookheimer,S.Y., and Mazziotta,J.C. (2000). Modulating emotional responses: effects of a neocortical network on the limbic system. Neuroreport *11*, 43-48.

Hariri,A.R., Mattay,V.S., Tessitore,A., Fera,F., Smith,W.G., and Weinberger,D.R. (2002a). Dextroamphetamine modulates the response of the human amygdala. Neuropsychopharmacology *27*, 1036-1040.

Hariri,A.R., Mattay,V.S., Tessitore,A., Fera,F., and Weinberger,D.R. (2003). Neocortical modulation of the amygdala response to fearful stimuli. Biological Psychiatry *53*, 494-501.

Hariri,A.R., Mattay,V.S., Tessitore,A., Kolachana,B., Fera,F., Goldman,D., Egan,M.F., and Weinberger,D.R. (2002b). Serotonin transporter genetic variation and the response of the human amygdala. Science *297*, 400-403.

Hariri,A.R., Tessitore,A., Mattay,V.S., Fera,F., and Weinberger,D.R. (2002c). The amygdala response to emotional stimuli: a comparison of faces and scenes. Neuroimage. *17*, 317-323.

Hart,A.J., Whalen,P.J., Shin,L.M., McInerney,S.C., Fischer,H., and Rauch,S.L. (2000). Differential response in the human amygdala to racial outgroup vs ingroup face stimuli. Neuroreport *11*, 2351-2355.

Harvey,P.O., Fossati,P., Pochon,J.B., Levy,R., Lebastard,G., Lehericy,S., Allilaire,J.F., and Dubois,B. (2005). Cognitive control and brain resources in major depression: an fMRI study using the n-back task. Neuroimage *26*, 860-869.

He,A.G., Tan,L.H., Tang,Y., James,G.A., Wright,P., Eckert,M.A., Fox,P.T., and Liu,Y. (2003). Modulation of neural connectivity during tongue movement and reading. Human Brain Mapping *18*, 222-232.

Hornak,J., Rolls,E.T., and Wade,D. (1996). Face and voice expression identification in patients with emotional and behavioural changes following ventral frontal lobe damage. Neuropsychologia *34*, 247-261.

Huettel,S.A., Song,A.W., and McCarthy,G. (2004). Functional magnetic resonance imaging. (Sunderland, MA.: Sinauer).

Irwin,W., Anderle,M.J., Abercrombie,H.C., Schaefer,S.M., Kalin,N.H., and Davidson,R.J. (2004). Amygdalar interhemispheric functional connectivity differs between the non-depressed and depressed human brain. Neuroimage *21*, 674-686.

Irwin,W., Davidson,R.J., Lowe,M.J., Mock,B.J., Sorenson,J.A., and Turski,P.A. (1996). Human amygdala activation detected with echo-planar functional magnetic resonance imaging. Neuroreport *7*, 1765-1769.

James,W. (1884). What is emotion? Mind *19*, 188-205.

Kanwisher,N., McDermott,J., and Chun,M.M. (1997). The fusiform face area: a module in human extrastriate cortex specialized for face perception. Journal of Neuroscience *17*, 4302-4311.

Keedwell,P.A., Andrew,C., Williams,S.C., Brammer,M.J., and Phillips,M.L. (2005). A double dissociation of ventromedial prefrontal cortical responses to sad and happy stimuli in depressed and healthy individuals. Biological Psychiatry *58*, 495-503.

Kennedy,S.H., Evans,K.R., Kruger,S., Mayberg,H.S., Meyer,J.H., McCann,S., Arifuzzman,A.I., Houle,S., and Vaccarino,F.J. (2001). Changes in regional brain glucose metabolism measured with positron emission tomography after paroxetine treatment of major depression. American Journal of Psychiatry *158*, 899-905.

Kerns,J.G., Cohen,J.D., MacDonald,A.W., III, Cho,R.Y., Stenger,V.A., and Carter,C.S. (2004). Anterior cingulate conflict monitoring and adjustments in control. Science *303*, 1023-1026.

Krolak-Salmon,P., Henaff,M.A., Isnard,J., Tallon-Baudry,C., Guenot,M., Vighetto,A., Bertrand,O., and Mauguiere,F. (2003). An attention modulated response to disgust in human ventral anterior insula. Annals of Neurology *53*, 446-453.

Kwong,K.K., Belliveau,J.W., Chesler,D.A., Goldberg,I.E., Weisskoff,R.M., Poncelet,B.P., Kennedy,D.N., Hoppel,B.E., Cohen,M.S., Turner,R., and . (1992). Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. Proceedings of the National Academy of Sciences of the United States of America *89*, 5675-5679.

Lane,R.D., Fink,G.R., Chau,P.M., and Dolan,R.J. (1997a). Neural activation during selective attention to subjective emotional responses. Neuroreport *8*, 3969-3972.

Lane,R.D., Reiman,E.M., Bradley,M.M., Lang,P.J., Ahern,G.L., Davidson,R.J., and Schwartz,G.E. (1997b). Neuroanatomical correlates of pleasant and unpleasant emotion. Neuropsychologia *35*, 1437-1444.

Lang,P.J. (1995). The emotion probe - studies of motivation and attention. American Psychologist *50*, 372-385.

Lang,P.J., Bradley,M.M., and Cuthbert,B.N. (2001). International affective picture system (IAPS): Instruction manual and affective ratings. Technical report A-5. (Gainesville, FL: The Center for Research in Psychophysiology, University of Florida).

Lang,P.J., Bradley,M.M., Fitzsimmons,J.R., Cuthbert,B.N., Scott,J.D., Moulder,B., and Nangia,V. (1998). Emotional arousal and activation of the visual cortex: an fMRI analysis. Psychophysiology *35*, 199-210.

Lazarus,R.S. (1982). Thought on the relations between emotion and cognition. American Psychologist *37*, 1024.

LeDoux,J.E. (2000). Emotion circuits in the brain. Annual Reviews in Neuroscience *23*, 155-184.

Leonard,C.M., Rolls,E.T., Wilson,F.A., and Baylis,G.C. (1985). Neurons in the amygdala of the monkey with responses selective for faces. Behavioral Brain Research *15*, 159-176.

Leppanen,J.M. (2006). Emotional information processing in mood disorders: a review of behavioral and neuroimaging findings. Current Opinion in Psychiatry *19*, 34-39.

Li,H., Albarracin,D., Wright,P., Brown,R.D., and Liu,Y., (2006). Evaluation proceduralization and its neural correlates. Unpublished manuscript, University of Florida, Gainesville, FL.

Liberzon,I., Taylor,S.F., Fig,L.M., Decker,L.R., Koeppe,R.A., and Minoshima,S. (2000). Limbic activation and psychophysiologic responses to aversive visual stimuli. Interaction with cognitive task. Neuropsychopharmacology *23*, 508-516.

Lieberman,M.D., Hariri,A., Jarcho,J.M., Eisenberger,N.I., and Bookheimer,S.Y. (2005). An fMRI investigation of race-related amygdala activity in African-American and Caucasian-American individuals. Nature Neuroscience *8*, 720-722.

Liotti,M., Mayberg,H.S., McGinnis,S., Brannan,S.L., and Jerabek,P. (2002). Unmasking disease-specific cerebral blood flow abnormalities: mood challenge in patients with remitted unipolar depression. American Journal of Psychiatry *159*, 1830-1840.

Logothetis,N.K., Pauls,J., Augath,M., Trinath,T., and Oeltermann,A. (2001). Neurophysiological investigation of the basis of the fMRI signal. Nature *412*, 150-157.

MacDonald,A.W., III, Cohen,J.D., Stenger,V.A., and Carter,C.S. (2000). Dissociating the role of the dorsolateral prefrontal and anterior cingulate cortex in cognitive control. Science *288*, 1835-1838.

Markowitsch,H.J. (1998). Differential contribution of right and left amygdala to affective information processing. Behavioural Neurology *11*, 233-244.

Matsuo,K., Glahn,D.C., Peluso,M.A., Hatch,J.P., Monkul,E.S., Najt,P., Sanches,M., Zamarripa,F., Li,J., Lancaster,J.L., Fox,P.T., Gao,J.H., and Soares,J.C. (2006). Prefrontal hyperactivation during working memory task in untreated individuals with major depressive disorder. Molecular Psychiatry.

Matsuo,K., Kato,C., Sumiyoshi,C., Toma,K., Duy Thuy,D.H., Moriya,T., Fukuyama,H., and Nakai,T. (2003). Discrimination of Exner's area and the frontal eye field in humans--functional magnetic resonance imaging during language and saccade tasks. Neuroscience Letters *340*, 13-16.

Mayberg,H.S. (1997). Limbic-cortical dysregulation: a proposed model of depression. Journal of Neuropsychiatry & Clinical Neurosciences *9*, 471-481.

Mayberg,H.S. (2003). Modulating dysfunctional limbic-cortical circuits in depression: towards development of brain-based algorithms for diagnosis and optimised treatment. British Medical Bulletin *65*, 193-207.

Mayberg,H.S., Brannan,S.K., Mahurin,R.K., Jerabek,P.A., Brickman,J.S., Tekell,J.L., Silva,J.A., McGinnis,S., Glass,T.G., Martin,C.C., and Fox,P.T. (1997). Cingulate function in depression: a potential predictor of treatment response. Neuroreport *8*, 1057-1061.

Mayberg,H.S., Brannan,S.K., Tekell,J.L., Silva,J.A., Mahurin,R.K., McGinnis,S., and Jerabek,P.A. (2000). Regional metabolic effects of fluoxetine in major depression: serial changes and relationship to clinical response. Biological Psychiatry *48*, 830-843.

Mayberg,H.S., Lozano,A.M., Voon,V., McNeely,H.E., Seminowicz,D., Hamani,C., Schwalb,J.M., and Kennedy,S.H. (2005). Deep brain stimulation for treatment-resistant depression. Neuron *45*, 651-660.

Mayberg,H.S., Silva,J.A., Brannan,S.K., Tekell,J.L., Mahurin,R.K., McGinnis,S., and Jerabek,P.A. (2002). The functional neuroanatomy of the placebo effect. American Journal of Psychiatry *159*, 728-737.

McKiernan,K.A., Kaufman,J.N., Kucera-Thompson,J., and Binder,J.R. (2003). A parametric manipulation of factors affecting task-induced deactivation in functional neuroimaging. Journal of Cognitive Neuroscience *15*, 394-408.

McNally,R.J. (2002). Disgust has arrived. Journal of Anxiety Disorders *16*, 561-566.

Menz,M.M., Neumann,J., Muller,K., and Zysset,S. (2006). Variability of the BOLD response over time: an examination of within-session differences. Neuroimage *32*, 1185-1194.

Merboldt,K.D., Fransson,P., Bruhn,H., and Frahm,J. (2001). Functional MRI of the human amygdala? Neuroimage *14*, 253-257.

Mesulam,M.M., and Mufson,E.J. (1982a). Insula of the old world monkey. I. Architectonics in the insulo-orbito-temporal component of the paralimbic brain. Journal of Comparative Neurology *212*, 1-22.

Mesulam,M.M., and Mufson,E.J. (1982b). Insula of the old world monkey. III: Efferent cortical output and comments on function. Journal of Comparative Neurology *212*, 38-52.

Miezin,F.M., Maccotta,L., Ollinger,J.M., Petersen,S.E., and Buckner,R.L. (2000). Characterizing the hemodynamic response: effects of presentation rate, sampling procedure, and the possibility of ordering brain activity based on relative timing. Neuroimage *11*, 735-759.

Mitterschiffthaler,M.T., Kumari,V., Malhi,G.S., Brown,R.G., Giampietro,V.P., Brammer,M.J., Suckling,J., Poon,L., Simmons,A., Andrew,C., and Sharma,T. (2003). Neural response to pleasant stimuli in anhedonia: an fMRI study. Neuroreport *14*, 177-182.

Morris,J.S., Frith,C.D., Perrett,D.I., Rowland,D., Young,A.W., Calder,A.J., and Dolan,R.J. (1996). A differential neural response in the human amygdala to fearful and happy facial expressions. Nature *383*, 812-815.

Morris,J.S., Ohman,A., and Dolan,R.J. (1998). Conscious and unconscious emotional learning in the human amygdala. Nature *393*, 467-470.

Morris,J.S., Ohman,A., and Dolan,R.J. (1999). A subcortical pathway to the right amygdala mediating "unseen" fear. Proceedings of the National Academy of Sciences of the United States of America *96*, 1680-1685.

Mufson,E.J., and Mesulam,M.M. (1982). Insula of the old world monkey. II: Afferent cortical input and comments on the claustrum. Journal of Comparative Neurology *212*, 23-37.

Narumoto,J., Yamada,H., Iidaka,T., Sadato,N., Fukui,K., Itoh,H., and Yonekura,Y. (2000). Brain regions involved in verbal or non-verbal aspects of facial emotion recognition. Neuroreport *11*, 2571-2576.

O'Doherty,J. (2004). Reward representations and reward-related learning in the human brain: insights from neuroimaging. Current Opinion in Neurobiology *14*, 769-776.

Ochsner,K.N., and Gross,J.J. (2005). The cognitive control of emotion. Trends in Cognitive Sciences *9*, 242-249.

Ochsner,K.N., Knierim,K., Ludlow,D.H., Hanelin,J., Ramachandran,T., Glover,G., and Mackey,S.C. (2004). Reflecting upon feelings: an fMRI study of neural systems supporting the attribution of emotion to self and other. Journal of Cognitive Neuroscience *16*, 1746-1772.

Ogawa,S., Lee,T.M., Kay,A.R., and Tank,D.W. (1990). Brain magnetic resonance imaging with contrast dependent on blood oxygenation. Proceedings of the National Academy of Sciences of the United States of America *87*, 9868-9872.

Ogawa,S., Tank,D.W., Menon,R., Ellermann,J.M., Kim,S.G., Merkle,H., and Ugurbil,K. (1992). Intrinsic signal changes accompanying sensory stimulation: functional brain mapping with magnetic resonance imaging. Proceedings of the National Academy of Sciences of the United States of America *89*, 5951-5955.

Ongur,D., and Price,J.L. (2000). The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. Cerebral Cortex *10*, 206-219.

Papez,J.W. (1937). A proposed mechanism of emotion. Archives of Neurology & Psychiatry *38*, 725-743.

Passer,M.W., and Smith,R.E. (2001). Psychology: frontiers and applications. (New York: McGraw-Hill).

Paulus,M.P., Feinstein,J.S., Castillo,G., Simmons,A.N., and Stein,M.B. (2005). Dose-dependent decrease of activation in bilateral amygdala and insula by lorazepam during emotion processing. Archives of General Psychiatry *62*, 282-288.

Pezawas,L., Meyer-Lindenberg,A., Drabant,E.M., Verchinski,B.A., Munoz,K.E., Kolachana,B.S., Egan,M.F., Mattay,V.S., Hariri,A.R., and Weinberger,D.R. (2005). 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. Nature Neuroscience *8*, 828-834.

Phan,K.L., Taylor,S.F., Welsh,R.C., Ho,S.H., Britton,J.C., and Liberzon,I. (2004). Neural correlates of individual ratings of emotional salience: a trial-related fMRI study. Neuroimage *21*, 768-780.

Phan,K.L., Wager,T., Taylor,S.F., and Liberzon,I. (2002). Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. Neuroimage. *16*, 331-348.

Phelps,E.A., O'Connor,K.J., Gatenby,J.C., Gore,J.C., Grillon,C., and Davis,M. (2001). Activation of the left amygdala to a cognitive representation of fear. Nature Neuroscience *4*, 437-441.

Phillips,M.L., Marks,I.M., Senior,C., Lythgoe,D., O'Dwyer,A.M., Meehan,O., Williams,S.C., Brammer,M.J., Bullmore,E.T., and McGuire,P.K. (2000). A differential neural response in obsessive-compulsive disorder patients with washing compared with checking symptoms to disgust. Psychological Medicine *30*, 1037-1050.

Phillips,M.L., Young,A.W., Senior,C., Brammer,M., Andrew,C., Calder,A.J., Bullmore,E.T., Perrett,D.I., Rowland,D., Williams,S.C., Gray,J.A., and David,A.S. (1997). A specific neural substrate for perceiving facial expressions of disgust. Nature *389*, 495-498.

Piggot,J., Kwon,H., Mobbs,D., Blasey,C., Lotspeich,L., Menon,V., Bookheimer,S., and Reiss,A.L. (2004). Emotional attribution in high-functioning individuals with autistic spectrum disorder: a functional imaging study. Journal of the American Academy of Child and Adolescent Psychiatry *43*, 473-480.

Pourtois,G., and Vuilleumier,P. (2006). Chapter 4 Dynamics of emotional effects on spatial attention in the human visual cortex. Progress in Brain Research *156*, 67-91.

Quirk,G.J., and Gehlert,D.R. (2003). Inhibition of the amygdala: key to pathological states? Annals of the New York Academy of Sciences *985*, 263-272.

Ramel,W., Goldin,P.R., Eyler,L.T., Brown,G.G., Gotlib,I.H., and McQuaid,J.R. (2006). Amygdala reactivity and mood-congruent memory in individuals at risk for depressive relapse. Biological Psychiatry.

Rauch,S.L., Whalen,P.J., Shin,L.M., McInerney,S.C., Macklin,M.L., Lasko,N.B., Orr,S.P., and Pitman,R.K. (2000). Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: a functional MRI study. Biological Psychiatry *47*, 769-776.

Roberson-Nay,R., McClure,E.B., Monk,C.S., Nelson,E.E., Guyer,A.E., Fromm,S.J., Charney,D.S., Leibenluft,E., Blair,J., Ernst,M., and Pine,D.S. (2006). Increased amygdala activity during successful memory encoding in adolescent major depressive disorder: an fMRI study. Biological Psychiatry.

Robinson,S., Windischberger,C., Rauscher,A., and Moser,E. (2004). Optimized 3 T EPI of the amygdalae. Neuroimage *22*, 203-210.

Rolls,E.T. (1999). The brain and emotion. (Oxford: Oxford University Press).

Rolls,E.T. (2000). The orbitofrontal cortex and reward. Cereb.Cortex *10*, 284-294.

Rolls,E.T., Inoue,K., and Browning,A. (2003). Activity of primate subgenual cingulate cortex neurons is related to sleep. Journal of Neurophysiology *90*, 134-142.

Rose,E.J., Simonotto,E., and Ebmeier,K.P. (2006). Limbic over-activity in depression during preserved performance on the n-back task. Neuroimage *29*, 203-215.

Royet,J.P., Plailly,J., Delon-Martin,C., Kareken,D.A., and Segebarth,C. (2003). fMRI of emotional responses to odors: influence of hedonic valence and judgment, handedness, and gender. Neuroimage *20*, 713-728.

Sabatinelli,D., Bradley,M.M., Fitzsimmons,J.R., and Lang,P.J. (2005). Parallel amygdala and inferotemporal activation reflect emotional intensity and fear relevance. Neuroimage *24*, 1265-1270.

Sander,D., Grafman,J., and Zalla,T. (2003). The human amygdala: an evolved system for relevance detection. Reviews in the Neurosciences *14*, 303-316.

Schienle,A., Stark,R., Walter,B., Blecker,C., Ott,U., Kirsch,P., Sammer,G., and Vaitl,D. (2002). The insula is not specifically involved in disgust processing: an fMRI study. Neuroreport *13*, 2023-2026.

Schwartz,C.E., Wright,C.I., Shin,L.M., Kagan,J., and Rauch,S.L. (2003). Inhibited and uninhibited infants "grown up": adult amygdalar response to novelty. Science *300*, 1952-1953.

Scott,S.K., Young,A.W., Calder,A.J., Hellawell,D.J., Aggleton,J.P., and Johnson,M. (1997). Impaired auditory recognition of fear and anger following bilateral amygdala lesions. Nature *385*, 254-257.

Serences,J.T. (2004). A comparison of methods for characterizing the event-related BOLD timeseries in rapid fMRI. Neuroimage *21*, 1690-1700.

Shapira,N.A., Liu,Y., He,A.G., Bradley,M.M., Lessig,M.C., James,G.A., Stein,D.J., Lang,P.J., and Goodman,W.K. (2003). Brain activation by disgust-inducing pictures in obsessive-compulsive disorder. Biological Psychiatry *54*, 751-756.

Sheline,Y.I., Barch,D.M., Donnelly,J.M., Ollinger,J.M., Snyder,A.Z., and Mintun,M.A. (2001). Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: an fMRI study. Biological Psychiatry *50*, 651-658.

Shima,K., and Tanji,J. (1998). Role for cingulate motor area cells in voluntary movement selection based on reward. Science *282*, 1335-1338.

Shin,L.M., Wright,C.I., Cannistraro,P.A., Wedig,M.M., McMullin,K., Martis,B., Macklin,M.L., Lasko,N.B., Cavanagh,S.R., Krangel,T.S., Orr,S.P., Pitman,R.K., Whalen,P.J., and Rauch,S.L. (2005). A functional magnetic resonance imaging study of amygdala and medial prefrontal

cortex responses to overtly presented fearful faces in posttraumatic stress disorder. Archives of General Psychiatry *62*, 273-281.

Shulman,G.L., Fiez,J.A., Corbetta,M., Buckner,R.L., Miezin,F.M., Raichle,M.E., and Petersen,S.E. (1997). Common blood flow changes across visual tasks: II. Decreases in cerebral cortex. Journal of Cognitive Neuroscience *9*, 648-663.

Shulman,R.G., Hyder,F., and Rothman,D.L. (2001). Cerebral energetics and the glycogen shunt: neurochemical basis of functional imaging. Proceedings of the National Academy of Sciences of the United States of America *98*, 6417-6422.

Siegle,G.J., Carter,C.S., and Thase,M.E. (2006). Use of FMRI to predict recovery from unipolar depression with cognitive behavior therapy. American Journal of Psychiatry *163*, 735-738.

Siegle,G.J., Steinhauer,S.R., Thase,M.E., Stenger,V.A., and Carter,C.S. (2002). Can't shake that feeling: event-related fMRI assessment of sustained amygdala activity in response to emotional information in depressed individuals. Biological Psychiatry *51*, 693-707.

Singer,T., Seymour,B., O'Doherty,J., Kaube,H., Dolan,R.J., and Frith,C.D. (2004). Empathy for pain involves the affective but not sensory components of pain. Science *303*, 1157-1162.

Soon,C.S., Venkatraman,V., and Chee,M.W. (2003). Stimulus repetition and hemodynamic response refractoriness in event-related fMRI. Human Brain Mapping *20*, 1-12.

Sprengelmeyer,R., Rausch,M., Eysel,U.T., and Przuntek,H. (1998). Neural structures associated with recognition of facial expressions of basic emotions. Proceedings of the Royal Society of London.Series B: Biological Sciences *265*, 1927-1931.

Sprengelmeyer,R., Young,A.W., Calder,A.J., Karnat,A., Lange,H., Homberg,V., Perrett,D.I., and Rowland,D. (1996). Loss of disgust - Perception of faces and emotions in Huntington's disease. Brain *119*, 1647-1665.

Sprengelmeyer,R., Young,A.W., Schroeder,U., Grossenbacher,P.G., Federlein,J., Buttner,T., and Przuntek,H. (1999). Knowing no fear. Proceedings of the Royal Society of London.Series B: Biological Sciences *266*, 2451-2456.

Stark,R., Schienle,A., Walter,B., Kirsch,P., Sammer,G., Ott,U., Blecker,C., and Vaitl,D. (2003). Hemodynamic responses to fear and disgust-inducing pictures: an fMRI study. International Journal of Psychophysiology *50*, 225-234.

Stefurak,T., Manhurin,R., and Soloman,D. (2001). Response specific regional metabolism changes with fluoxetine treatment in depressed Parkinson's patients. Movement Disorders *16*, S39.

Surguladze,S., Brammer,M.J., Keedwell,P., Giampietro,V., Young,A.W., Travis,M.J., Williams,S.C., and Phillips,M.L. (2005). A differential pattern of neural response toward sad versus happy facial expressions in major depressive disorder. Biological Psychiatry *57*, 201-209.

Talairach,J., and Tournoux,P. (1988). Co-Planar Stereotaxic Atlas of the Human Brain. Thieme Medical Publishers, Inc.).

Taylor,S.F., Liberzon,I., Fig,L.M., Decker,L.R., Minoshima,S., and Koeppe,R.A. (1998). The effect of emotional content on visual recognition memory: a PET activation study. Neuroimage *8*, 188-197.

Taylor,S.F., Phan,K.L., Decker,L.R., and Liberzon,I. (2003). Subjective rating of emotionally salient stimuli modulates neural activity. Neuroimage *18*, 650-659.

Tessitore,A., Hariri,A.R., Fera,F., Smith,W.G., Chase,T.N., Hyde,T.M., Weinberger,D.R., and Mattay,V.S. (2002). Dopamine modulates the response of the human amygdala: a study in Parkinson's disease. Journal of Neuroscience *22*, 9099-9103.

Tessitore,A., Hariri,A.R., Fera,F., Smith,W.G., Das,S., Weinberger,D.R., and Mattay,V.S. (2005). Functional changes in the activity of brain regions underlying emotion processing in the elderly. Psychiatry Research *139*, 9-18.

Vogt,B.A. (2005). Pain and emotion interactions in subregions of the cingulate gyrus. Nature Reviews.Neuroscience *6*, 533-544.

Vuilleumier,P., Armony,J.L., Driver,J., and Dolan,R.J. (2001). Effects of attention and emotion on face processing in the human brain: an event-related fMRI study. Neuron *30*, 829-841.

Wagner,G., Sinsel,E., Sobanski,T., Kohler,S., Marinou,V., Mentzel,H.J., Sauer,H., and Schlosser,R.G. (2006). Cortical inefficiency in patients with unipolar depression: an event-related FMRI study with the Stroop task. Biological Psychiatry *59*, 958-965.

Wang,A.T., Dapretto,M., Hariri,A.R., Sigman,M., and Bookheimer,S.Y. (2004). Neural correlates of facial affect processing in children and adolescents with autism spectrum disorder. Journal of the American Academy of Child and Adolescent Psychiatry *43*, 481-490.

Whalen,P.J., Bush,G., McNally,R.J., Wilhelm,S., McInerney,S.C., Jenike,M.A., and Rauch,S.L. (1998a). The emotional counting Stroop paradigm: a functional magnetic resonance imaging probe of the anterior cingulate affective division. Biological Psychiatry *44*, 1219-1228.

Whalen,P.J., Rauch,S.L., Etcoff,N.L., McInerney,S.C., Lee,M.B., and Jenike,M.A. (1998b). Masked presentations of emotional facial expressions modulate amygdala activity without explicit knowledge. Journal of Neuroscience *18*, 411-418.

Wicker,B., Keysers,C., Plailly,J., Royet,J.P., Gallese,V., and Rizzolatti,G. (2003). Both of us disgusted in my insula. The common neural basis of seeing and feeling disgust. Neuron *40*, 655-664.

Wilson,J.L., and Jezzard,P. (2003). Utilization of an intra-oral diamagnetic passive shim in functional MRI of the inferior frontal cortex. Magnetic Resonance in Medicine *50*, 1089-1094.

Winston,J.S., O'Doherty,J., and Dolan,R.J. (2003). Common and distinct neural responses during direct and incidental processing of multiple facial emotions. Neuroimage *20*, 84-97.

Winston,J.S., Strange,B.A., O'Doherty,J., and Dolan,R.J. (2002). Automatic and intentional brain responses during evaluation of trustworthiness of faces. Nature Neuroscience *5*, 277-283.

Wright,C.I., Fischer,H., Whalen,P.J., McInerney,S.C., Shin,L.M., and Rauch,S.L. (2001). Differential prefrontal cortex and amygdala habituation to repeatedly presented emotional stimuli. Neuroreport *12*, 379-383.

Wright,P., He,G., Shapira,N.A., Goodman,W.K., and Liu,Y. (2004). Disgust and the insula: fMRI responses to pictures of mutilation and contamination. Neuroreport *15*, 2347-2351.

Wright,P., and Liu,Y. (2005). Neutral faces activate the amygdala during identity matching. Neuroimage *29*, 628-636.

Young,A.W., Aggleton,J.P., Hellawell,D.J., Johnson,M., Broks,P., and Hanley,J.R. (1995). Face processing impairments after amygdalotomy. Brain *118 (Pt 1)*, 15-24.

Zajonce,R.B. (1980). Feeling and thinking: Preferences need no inferences. American Psychologist *35*, 151-175.

BIOGRAPHICAL SKETCH

Paul Wright was born in Dover, England, in 1974. He received his secondary education at the Ashcombe School, Dorking, where he obtained 'A'-levels in chemistry, physics, and mathematics. After spending a year as a volunteer teaching secondary school science in rural Kenya, Paul enrolled at the University of Southampton, England, to study medicine. He completed a study on the rat hippocampus in his fourth year, but on resuming clinical training decided against life on the wards. He was awarded a Bachelor of Medical Science in 1997, and worked on studies of lipid metabolism as a technician in the Institute for Human Nutrition at his *alma mater*. He joined the University of Florida's Interdisciplinary Program in Medical Science in 2001, where his interest in the brain reasserted itself. He has since published two first author articles on the functional neuroimaging of emotion. After graduating in December 2006, Paul plans to complete some articles and analysis in the lab of Yijun Liu, and will then pursue post-doctoral employment in England.